THE INFLUENCE OF CONTEXT AND STRATEGY ON SPATIAL TASK PERFORMANCE

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Abstract

This thesis examines naturally occurring variability in the performance of spatial tasks in order to shed light on the neurobiology that underpins human experience. It tests the theory that differences in performance of spatial tasks are an emergent property of differences in how contextual information is interpreted and the strategy implemented during task performance. Results indicate that enhanced performance accuracy in males may reflect the use of a more topographically tuned strategy rather than better spatial ability than females *per se*. Males and females may have different pressures leading to tendencies to rely more often on particular strategies, but this does not mean that generally one group is better than the other. Differential recruitment of lateral and medial entorhinal cortex and the nature of the information processed therein and in afferent regions of hippocampus may be what drives the differences in spatial accuracy and strategy implementation between males and females.

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ANOVA – analysis of variance
BOLD – blood oxygen level dependent
CA – cornu ammonis
EC – entorhinal cortex
EEG - electroencephalography
ELISA – enzyme linked immune salivary assay
ERP – event related potential
EP – evoked power
FDR – false discovery rate
fMRI – functional magnetic resonance imaging
IP – induced power
ITC – inter-trial coherence
LEC – lateral entorhinal cortex
LTP – long term potentiation
M - mean
MEC – medial entorhinal cortex
MRI – magnetic resonance imaging
MRS – magnetic resonance spectroscopy
MWT – Morris water task
OL – object location
PPC – posterior parietal cortex
PPA – parahippocampal place area
PHC – parahippocampal cortex
RSC – retrosplenial cortex
SEM – standard error of the mean
TSE – temporal spectral evolution

Chapter One

General Introduction

"All this happened"

-- Kurt Vonnegut, Slaughterhouse Five

The quantification of memory can be difficult given the ephemeral nature of experience. There are some types of memory (e.g., memory for events or facts) that can be readily quantified, but there are others whose quantification can be more problematic. For example, episodic memories incorporate a myriad of factors including specifics about time and place, but also emotional valence, attended stimuli or the weighting of factors within the event. These factors will probably differ for each subject as a result of past experiences. Conversely, distance, displacement, and movement, important elements of spatial navigation learning and memory, can all be quantified using established methods with relative confidence. If an investigator could quantify experiences in the same way they could quantify distance, considerable gains could be made in our understandings of experience and memory. Fortunately for memory investigations, spatial tasks rely on many of the same neural regions that underlie episodic memory processes (e.g., the hippocampal complex) in a broad range of situations (Burgess, Maguire, & O'Keefe, 2002). As a result, the assessment of spatial task performance provides a lens by which episodic memory and the machinery that underlies complex processes can be examined in a quantifiable way.

This thesis examines naturally occurring variability in the performance of spatial tasks in an attempt to shed light on the neurobiology that underpins human experience. In doing so, it tests the theory **that differences in performance of spatial tasks are**

emergent properties of differences in how contextual information is interpreted and the strategy implemented during task performance.

Navigation is a complex phenomenon comprised of multiple processes including the determination of one's current location, selection of a goal and calculation of a trajectory or set of trajectories to achieve the goal. The agents must also update their location as they move toward their goal, recalculating their current position within their representation of the environment (i.e., a cognitive map). In addition, detours are often required in the real world, meaning that sufficient flexibility must be maintained to incorporate planning of alternate routes within a single outing. Though complicated, even insects, animals with relatively simple neural systems, are capable of navigating accurately through complex environments (Collett & Collett, 2002).

The way an animal moves through a complex environment can be contingent on not just its goals, but also the richness of its representation of the space. When searching for a target within a set area particular strategies become readily apparent (Janus, 2014). For example, when searching a circular enclosure for a hidden target, naïve animals will initially spend most of their time tracing the wall and slowly moving away from the edge, which is referred to as thigmotaxis. Once the animal has learned the nature of the task, they may begin to "chain" wherein the animal travels along the edge and sporadically moves away from the wall at the approximate distance the target is placed from the wall. An alternative to this is to search the area in either a random fashion or by creating a geometric pattern and following it. Once the animal has learned the location of a goal, the search tends to become more direct wherein they either target a smaller area near to the target location or travel directly to the goal site.

There are also different frames of reference that can be used to represent spatial information. For example, an egocentric framework defines positions in the environment relative to the axes of the subject's body (e.g., the target is to my left). An alternate frame of reference, referred to as allocentric, defines the space using various positions relative to each other, independent of the subject's body position. Though often thought of as independent, successful navigation requires the integration of both of these spatial coding systems.

In addition to determining goals and trajectories, accurate navigation also requires maintaining spatial information in short-term memory storage (Bird & Burgess, 2008). There is general consensus throughout the literature that the hippocampus contributes to the performance of spatial tasks, but the contributions of working memory on task performance are thought to depend on areas outside the hippocampus, such as prefrontal cortex. For example, dorsolateral prefrontal cortex has been implicated in the maintenance of target locations in working memory on visuo-spatial tasks in nonhuman primates (Wimmer, Nykamp, Constantinidis, & Compte, 2014). In humans, the posterior parietal cortex as well as the dorsolateral prefrontal cortex have been implicated in spatial working memory tasks such as the Corsi block task (van Asselen et al., 2006). The connectivity between hippocampus and prefrontal cortex is important for the performance of spatial tasks (Spellman et al., 2015) and this is likely a result of the need to integrate spatial knowledge with the development/maintenance of a plan. In short, the expedient performance of these types of tasks requires the subject be able to track their current position within a sequence of behaviours, which is similar to the process of path integration. This may seem relatively straightforward, but it is a complex process that incorporates multiple facets of intelligence, memory, and attention.

Path integration is the process of extrapolating the movement between points so that an organism's current position is updated relative to cues such as environmental landmarks (i.e., large, unique environmental features) or egocentric position in space (McNaughton, Battaglia, Jensen, Moser, & Moser, 2006). Though path integration could be considered egocentric, it requires precise binding of the egocentric representation of body position with the distal environmental cues in order to be accurate. Other strategies for successful navigation include sun-compass navigation in birds (Guilford & Taylor, 2014) or reference to constellations of stars in the night sky amongst nocturnal species such as the Namib Desert spider (*Leucorchestris arenicola*)(Nørgaard, 2005). Similarly, some organisms possess a suite of sensory organs designed to detect changes in particular sets of stimuli (be they magnetic, olfactory, visual, etc.) and use gradations in sensory cues to derive compasses that can be used to orient the organism toward a desired target or location under variable conditions (for review see, Phillips, Schmidt-Koenif, & Mulheim, 2006).

In most vertebrates, several neural regions are important for supporting navigational abilities, though the hippocampus is often thought to be the most important. The hippocampal formation is comprised of several distinct subregions including the hippocampus proper - dentate gyrus and cornu ammonis (CA) fields 1, 2, and 3, the subiculum, presubiculum, parasubiculum, and entorhinal cortex (EC)(Amaral & Witter, 1989). Though each subfield performs a unique set of information processing steps, the output of the hippocampus requires integration of the disparate types of information into a population code.

In terms of memory function, it appears that the dentate gyrus and CA3 regions of hippocampus support pattern separation (Bakker, Kirwan, Miller, & Stark, 2008) which is

the ability to discriminate between cues with overlapping elements. Conversely, CA1 appears to support pattern completion, a process wherein a complete memory is retrieved from partial stimuli. Cells in CA2 appears to encode social and contextual information while operating within the same spatial framework as other hippocampal subregions as indicated by global remapping, but no changes in overall activity levels in CA2 following exposure to social or novel stimuli (Alexander et al., 2016). Further support for the hypothesis that CA2 is encoding spatial, emotional, or temporal aspects of memory is generated by the observation that the activity of cells within CA2 are not stable across time – unlike the activity of cells within other hippocampal subfields, and that activity patterns in CA2 become progressively more dissimilar across time (Mankin, Diehl, Sparks, Leutgeb, & Leutgeb, 2015). Cells throughout the parahippocampal complex exhibit place dependent activity. Place cell activity is linked to a unique place in the environment and, although a single cell can be active in multiple environments its specific place field generally does not maintain the same topographical relationship with other place cells across environments (Barry & Burgess, 2014). Place cells are thought to participate in creating a cognitive map of the environment (Derdikman & Moser, 2010). CA1 maintains reciprocal connections with the subicular cortices, which possess distinct populations of cells called head direction cells that fire when the animal is oriented in a particular perceived direction (Boccara et al., 2010; Jeffery, 2007). The subiculum reconnects with hippocampus via the entorhinal cortex (EC) – the largest afferent to hippocampus (Boccara et al., 2010; Canto, Wouterlood, & Witter, 2008).

The EC is divided into two subregions, lateral and medial EC, based on function, location, cytoarchitecture, and projections (Canto et al., 2008). The lateral EC (LEC), which is also referred to as Brodmann's Area 28a, processes stimuli from various

modalities including somatosensory, visual, and olfactory regions and, unlike most other parahippocampal regions, is only loosely modulated by theta and the metric information it encodes is largely derived from the local cues in the environment, at least in rodents (Knierim & Neunuebel, 2015). The medial EC (MEC), which is also referred to as Brodmann's Area 28b, encodes spatial information through populations of grid, boundary, and head direction cells (for review see (Knierim & Neunuebel, 2015) and can be argued to be responding to environmental cues such as spatial geometry, direction, and distance (Knierim & Neunuebel, 2015). Knierim and Neunuebel (2015) outline the importance of competing inputs to hippocampal targets from LEC and MEC. When complexes of cellular firing are sent from EC to DG, the information is transcribed into a DG code in such a way that it is more likely to be separated into distinct events (i.e., pattern separation). Conversely, DG and EC inputs to CA3 are more likely to invoke a process called pattern completion. Contrary to earlier reports, Knierim and Neunuebel (2015) observed that when the information is sent to CA1 – which is also a major output to EC, pattern separation and pattern completion can both be observed within the same populations of cells. This is especially interesting when one considers that the collection of information that has been sent by these upstream structures is feeding back into EC and DG. When the system is challenged with competing signals, such as by the rotation of cues or an apparatus, cells within each subregion react accordingly, except in CA1, where individual cells reportedly represent information that derives from both LEC and MEC. There are important implications of this distinction as well as the preservation of MEC and LEC signals as they exit the hippocampus, especially for the performance of tasks that rely on the effective use of global and/or local cues for solving spatial problems.

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Place and head directional cells would be ineffective without the ability to estimate distance. MEC grid cells exhibit firing in a hexagonal grid-like pattern that is consistent across environments (Boccara et al., 2010). The spacing of the grid increases along the dorsal-ventral axis of the MEC (Brun et al., 2008). Grid cells provide important spatial metric input to other cells types for use in navigating. Additionally, there appears to be specialization of function by grid cell anatomical type whereby MEC pyramidal cells are specialized for refining within-entorhinal computations thereby maximizing spatial periodicity and path integration and MEC stellate cells support firing patterns associated with the orthogonalization of memory processes through entorhinal-hippocampal connectivity (Sasaki, Leutgeb, & Leutgeb, 2015). The first place the distinct information being processed by LEC and MEC is combined is in the hippocampus (Derdikman & Moser, 2010). Together, these cell types comprise key components of the hippocampal spatial navigation system, but other systems capable of processing spatial information also exist.

Cortical Components of Navigational Systems. The parietal cortex is important for various somatosensory and motor functions. Unique subregions support distinct behavioural abilities, but the most important for the current thesis is the posterior parietal cortex – which sits at a juncture of visual, motoric, and memory/spatial areas of the brain (Andersen, Snyder, Bradley, & Xing, 1997). The posterior parietal cortex appears to be preferentially activated by spatial information and exhibits strong, bidirectional activity with medial temporal lobe regions. One such region, the parahippocampal place area (PPA), which sits at the boundary of the posterior parahippocampal cortex and anterior lingual gyrus is strongly responsive to views of scenes (Epstein, 2008). It is likely the case that PPA encodes both geometric and non-geometric properties of a scene given that

is does not exhibit altered firing rates when shown the same scene with differing numbers of objects in conjunction with observed patterns of connectivity to regions processing physical characteristics such as texture or color (Epstein, 2008). Whereas the hippocampus encodes individual object locations within a scene as unique traces, the PPA does not alter its response when objects within the scene are added or removed, implying that PPA treats the entire scene as a whole representation rather than a collection of individual components. There does not appear to be a mnemonic component to PPA based on Epstein's (2008) observation that it is equally responsive to familiar and novel scenes. PPA is important for the process of wayfinding (Epstein, 2008). Posterior cerebral artery strokes often result in damage to PPA and wayfinding is negatively affected in this patient population. The deficit appears to be in recognizing or processing information about one's own position or orientation in the world because the ability to use small cues like landmarks is spared (Epstein, 2008).

While it is often regarded as a perceptual system, the PPC is also an action system. The parietal cortex is a key component of the dorsal or the "where/how" pathway of visual information processing (Goodale & Milner, 1992). In primates, posterior parietal cortex integrates information from various systems to create representations that inform multiple abilities (e.g., visual, motoric, haptic responses) (Andersen, 1997). Individuals who have compromised ventral visual paths, but intact posterior parietal cortices (PPCs) often exhibit preserved movement through space which implies that the PPC is an action system as well as a perceptual one (Goodale & Milner, 1992). There is also strong evidence for the role of PPC in the execution of spatially aware plans amongst rodents with PPC lesions (Whitlock et al., 2008). The PPC is important for incorporating

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information across multiple modalities in order to successfully interpret information about position and movement.

Findings such as those of Epstein and colleagues have led some to suggest that PPC, along with PPA, is encoding information from an egocentric view by defining the spatial layout relative to large fixed surface. Further, given the types of impairments observed consequent to damage to PPA as well as PPC, it has also been argued that information about body position is integrated with information derived from the environment (Whitlock, Sutherland, Witter, Moser, & Moser, 2008).

Complex navigation in human subjects

Obviously, differing amounts of experience or expertise can influence navigational ability. For example, London taxi drivers undergo rigorous spatial navigation training in order to become licensed. Consequently, they provide a unique opportunity to examine how extreme navigational expertise influences the neuroanatomy that underlies spatial abilities. The volume of the anterior hippocampus is reduced in London taxi drivers, but gray matter in the middle posterior portion of hippocampus increases proportionally with the amount of experience or length of time working as a taxi driver (Maguire, Woollett, & Spiers, 2006). Functional magnetic resonance imaging (fMRI) studies have revealed increased blood oxygen level dependent (BOLD) activity localized to the posterior hippocampus, the uncus in particular, in experienced taxi drivers and during accurate navigation amongst the general public who do not possess enhanced posterior hippocampal gray matter volume (Hartley, Maguire, Spiers, & Burgess, 2003).

Evidence of the role of hippocampus during the construction of a remembered context or scene is clear, despite some anomalies within the literature, involvement of the

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hippocampus is spatial processing and some forms of memory are reported across all species that have a hippocampus. Interestingly, the hippocampus has also been implicated in the construction of imagined scenes. Damage to the hippocampus results in an inability to visualize imagined scenes (Hassabis, Kumaran, Vann, & Maguire, 2007; Hassabis & Maguire, 2009). This observation has led some to postulate that the role of the hippocampus is to construct spatially coherent scenes thereby providing efficient storage of episodic and episodic-like imaginary information into discrete scenes (Maguire & Mullally, 2013; Mullally & Maguire, 2014).

Patterns of BOLD responses that can discriminate between populations are not limited to experts and non-experts. The implementation of a particular strategy is characterized by unique patterns of BOLD activity. For example, when subjects are able to rely on a spatial or a cue-based strategy, differential BOLD activity is observed in the hippocampus and caudate nucleus associated with each strategy, respectively (Bohbot, Lerch, Thorndycraft, Iaria, & Zijdenbos, 2007; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). Patterns of differential activity are not limited to distinct strategy selection, but also task phase. Memory encoding is associated with increased BOLD activity in left medial temporal lobe. Conversely, when memory for an OL pair is being recalled, activation is biased toward the right hemisphere (Owen, Milner, Petrides, & Evans, 1996).

An individual's propensity to use a particular strategy is, at least, partially dependent on their underlying neuromorphology. For example, the altered hippocampi of London taxi drivers are accompanied by a huge capacity to learn or retain spatial information. The counterpoint of these expert navigators may be individuals with developmental topographic disorientation (DTD). DTD individuals have a lifelong history of becoming

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lost, even in environments as familiar as their own neighborhoods or homes multiple

times per week, despite falling within normal ranges on all other aspects of cognitive

function (Iaria & Barton, 2010). In the first reported cases of DTD, no BOLD activity of

the hippocampal complex or retrosplenial cortex (RSC) was observed, whereas activation

increased in both regions was observed amongst all control subjects (Iaria, Bogod, Fox, &

Barton, 2009). One possible interpretation of this result is that DTD individuals rely on

neural regions outside medial temporal lobe when performing spatial tasks. Conversely, it

is possible that information processing within parahippocampal regions is compromised

in such a way that the coherence of the signal is degraded.

Further, judicious use of a particular strategy can also influence task performance.

For example, if someone was using landmarks to navigate and they select permanent,

highly visible structures, they are likely to be successful in returning to a previously

learned location. Conversely, an individual relying on landmarks that are impermanent,

such as other cars in a parking lot in order to remember where they themselves have

parked, is unlikely to be successful when attempting to return to the same location as the

cues are likely gone. Using fMRI, Auger and colleagues (2012) were able to determine

that both the parahippocampal- and retrosplenial-cortices (RSC) are responsive to the

presentation of landmarks, but that the RSC responds to only the most permanent of

landmarks. A permanent landmark would be some stable characteristic of the

environment (e.g., the shape and size of a building). Conversely, an impermanent

landmark is one that could be easily moved (e.g., a small or transient object) or attending

to a particular attribute that is relatively unstable (e.g., season changes in foliage color). A

comparison of strong and weak navigators revealed that RSC activity was reduced in

weak navigators and this was accompanied by difficulty identifying the most permanent

landmarks (Auger, Mullally, & Maguire, 2012). Physiological observations of differential activity during accurate and inaccurate navigation (Maguire et al., 2003) is not just limited to fMRI data. A positive correlation between accurate trials on a virtual navigation task and increased theta activity has been observed using scalp-recorded electroencephalography (EEG) (White, Congedo, Ciorciari, & Silberstein, 2012).

The influence of strategy selection can be missed if an experimenter fails to incorporate methods of controlling strategy selection (e.g., explicit instruction/restriction of information) or altering task demands. In an elegant series of studies, Saucier and colleagues revealed that the purported male superiority in spatial abilities can be attributed, at least in part, to differential strategy implementation (Saucier et al., 2002). More specifically, individuals were required to solve a task using either Euclidean (i.e., allocentric) or landmark based instructions, females who were required to use Euclidean instructions performed more poorly than females using landmark instructions and males using both landmark and Euclidean instructions, regardless of whether the task required real world navigation or a tabletop adaptation (Saucier et al., 2002). Careful tweaks in method such as these illustrate why sex differences are so inconsistently reported in the scientific literature.

Further, the nature of the task may influence the neural regions recruited during the performance of the task. For example, tasks assessing encoding of space on a peripersonal task generate increased BOLD signals along the intraparietal sulcus, the supramarginal gyrus, and premotor areas (Brozzoli, Gentile, Petkova, & Ehrsson, 2011). A peripersonal spatial task similar to the Morris water task that required subjects to locate a hidden target using their finger on a tabletop apparatus failed to failed to yield a sex difference (Köppen et al., 2013), possibly because there are fewer sex differences in the neural regions that

are reported to support performance on tasks assessing representations of peripersonal space (i.e., the space within arm's reach). The use of virtual tasks that rely on the perception of movement through visual motion tend to be more consistent with the sex differences reported in real world movement tasks in animals (Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005), but it is also possible that these tasks do not approximate true, full body movement through space. This criticism has been addressed through a series of intracranial EEG recordings performed on epileptic patients who exhibit patterns of activity during virtual navigation tasks that are consistent with what is observed amongst nonhuman animals during the performance of analogous spatial tasks that require movement through the real world (Ekstrom et al., 2005; Ekstrom et al., 2003; Lega, Jacobs, & Kahana, 2012).

Humorous anecdotes about differences in how males and females navigate are old hat and, as undesirable as it may be to advocates of complete gender equality of ability, these purported differences have empirical support (Sherry & Hampson, 1997). Traditional definitions of successful task performance such as absolute target accuracy for cardinal directions consistently reveal a male advantage. However, when landmarks are available, thereby allowing for the implementation of a more egocentric strategy, the advantage is reduced or disappears entirely (Saucier et al., 2002). In lieu of explicit instruction, males tend to implement strategies that involve the use of cardinal directions whereas females use landmarks to develop a strategy to successfully solve a task (Andersen, Dahmani, Konishi, & Bohbot, 2012) (Rahman, Andersson, & Govier, 2005). Conversely, superior performance by females is observed on object location memory tasks (e.g., Object Location Memory Task)(Silverman, Choi, & Peters, 2007), though some debate regarding the influence of verbal memory persists (Choi & L'Hirondelle, 2005). These propensities

appear to be preserved across mammals (Clint, Sober, Garland, & Rhodes, 2012), which implies that the differences are an innate feature of biological sex (or reflect very general constancies in the environment-biological sex interactions) and not acquired due entirely to gender-related experiential factors or differences in verbal memory (Luine, Jacome, & MacLusky, 2003; Saucier, Shultz, Keller, Cook, & Binsted, 2008).

There have always been inconsistencies in reports of sex differences in the scientific literature (Caplan, MacPherson, & Tobin, 1985). Fluctuations in performance have been attributed to strategy selection (Saucier et al., 2002), circulating hormone levels (Luine, 2014), or experience (Uttal et al., 2013). It is possible that the state of the literature on sex differences is confounded by the fact that researchers are using spatial abilities as a metric to assess different primary outcomes such as those listed above and that if the scope of meta-analyses of sex differences were restricted to those that aligned along a primary outcome (i.e., use the independent variable – such as circulating hormone levels – as the primary measure) a more consistent literature would emerge. Additionally, the effect sizes of most sex differences tend to be small to medium and the magnitude of these differences change along subject variables such as age, experience, or culture as well as the type of spatial task performed (Voyer et al, 1995).

On average, a male advantage is observed during the performance of spatial tasks (Jonasson, 2005), though a female advantage is consistently observed for object-location types of tasks (Levy et al., 2005). Further, some tasks, such as radial maze, find no sex differences (Levy et al., 2005). The variability in results likely reflects the differences in spatial task design and implementation in conjunction with individual differences in hormone status, age, and experience.

One finding within the body of literature on sex differences in spatial abilities are the propensities for males and females exhibit preferential reliance on geometric and contextual cues, respectively. These cue types may also represent the differential contributions of LEC and MEC (Knierim & Neunuebel, 2015). It is possible that sexually dimorphic EC underlies the sex specific trends for the implementation of particular strategies, and thus the sex differences observed during the performance of spatial tasks.

Roof, Zhang, Glasier, and Stein (1993) reported that unilateral lesions of entorhinal cortex elicited impairments on Morris water task performance in male rats, but not females. Pictorial representations of the lesions indicate that LEC may have been spared, providing additional support for the sex difference in reliance on MEC and LEC in males and females, respectively. Increased gray matter volume in entorhinal cortex has been found for males relative to females, though the authors did not compare medial and lateral regions of EC (Good et al., 2001). Garcia-Falgueras et al. (2006) also report increased EC volume in males without distinguishing between MEC and LEC. It seems probable that the subdivisions of EC are either sexually dimorphic and likely exhibit dissociable differences in patterns of activation during spatial task performance given the reported differences in overall EC volume. It seems probable that the subdivisions of EC are either sexually dimorphic and likely exhibit dissociable differences in patterns of activation during spatial task performance given the reported differences in overall EC volume.

Circulating levels of gonadal steroids such as testosterone and estrogen are reported to alter spatial task performance within each sex (Andreano & Cahill, 2009; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Gunturkun, 2000). The influence of hormones on the organization of neural structures (i.e., organization effects of sex hormones) tend to be robustly supported (Berenbaum & Beltz, 2011), but the immediate,

or activational, effects of circulating levels of hormones have received only partial support (for example see, Driscoll et al., 2005; Kimura & Hampson, 1994; Luine et al., 2003), but see (Liben et al., 2002). Circulating hormone levels are a likely contributor to the inconsistency with which sex differences are reported.

Hormone dependent fluctuations in both hippocampal neurogenesis (Pawluski, Brummelte, Barha, Crozier, & Galea, 2009) and volume (Protopopescu et al., 2008) have also been reported providing a potential mechanism for the fluctuations in strategy that fluctuate across the estrus cycle (Korol, Malin, Borden, Busby, & Couper-Leo, 2004). One aim of this thesis is to assess the hypothesis that *differences in target accuracy and attended navigational cues/strategy selection will be mediated by circulating hormone levels and biological sex.*

The study of sex differences is important because it provides insight into how spatial problems can be solved using different strategies. Sex differences in the performance of spatial tasks also provide a naturally occurring opportunity to study how information can be processed in related, but distinct ways. Many dementia patients arrive at their primary health care provider with complaints of disorientation or because they have become lost in previously familiar locales (Ballard, Mohan, Bannister, Handy, & Patel, 1991; Boise, Camicioli, Morgan, Rose, & Congleton, 1999), leading to a loss of independence and, ultimately, the need for institutionalization. By describing characteristics of the navigation system in healthy individuals, it is possible that strategies that rely on neural regions resistant to dementia-related breakdown can be identified and taught to individuals at risk of developing dementia, thereby prolonging their independence and reducing stressors to care providers.

Electrophysiological properties of the navigation system

The most common frequency of electrophysiological activity associated with neural activity in the hippocampal formation is theta (Buzsaki, 2002). Theta activity ranges from 1 – 7 Hz in humans (Hanslmayr & Staudigl, 2014), though ranges are species-specific (Buzsaki, 2005). At least part of the hippocampal theta cycle is generated from the medial septum and specific brain stem nuclei and is thought to be partially dependent on cholinergic inputs to the hippocampal complex (Fisahn, Pike, Buhl, & Paulsen, 1998; Lee, Chrobak, Sik, Wiley, & Buzsaki, 1994). Interestingly, there appear to be two separate contributors to hippocampal theta. The first, often referred to as type 1 theta, is not cholinergic dependent as indicated by the persistence of hippocampal theta activity following the administration of anticholinergic drugs. The second, type two theta is blocked by the administration of anticholinergic drugs (Vertes & Kocsis, 1997). Theta activity has been associated with a myriad of cognitive function, though all those included are characterized by voluntary, purposive behaviour (Buzsaki, 2005). Theta activity is highly correlated with the activity of the animal during navigation (McNaughton et al., 2006; Watrous, Fried, & Ekstrom, 2011). The amplitude of theta activity corresponds to the rate of movement, with quicker movement associated with higher amplitudes and frequencies of theta than slower traversals of the space. Likewise, when an animal reaches a decision point during task performance increased electrophysiological activity is observed across the theta bandwidth (Benchenane et al., 2010). In some circumstance, theta appears to be modulated by the activity of large populations of hippocampal pyramidal cells (Vertes & Kocsis, 1997). The ability of environmental stimuli to modulate hippocampal theta is important given the critical information contained in this signal for making accurate approximations about positional displacement during

voluntary movement or exploration and intra-hippocampal modulation of theta activity

provides a mechanism by which the parahippocampal system is able to self-regulate this

information.

Increases in power in the theta, and to a lesser extent gamma (25-40 Hz), bandwidths

at the time of encoding are associated with improved recall (Lopez-Azcarate et al., 2013).

In addition to the overall electrophysiological power, the coherence of activity across

neural regions has important implications. The phase of theta activation is also related to

how well information is encoded and remembered. More precisely, when oscillations are

"in phase" or synchronized across neural regions, memory performance improves (Fell &

Axmacher, 2011). Further, when events occur in the trough of the theta cycle, memory

performance is impaired (Nokia, Waselius, Mikkonen, Wikgren, & Penttonen, 2015). In

addition to synchronization within a bandwidth, synchronized activity across bandwidths

also impacts how stimuli are encoded. For example, coherent oscillatory activity across

gamma and theta in hippocampus and rhinal sulcus regardless of absolute power is

associated with improved memory formation (Fell et al., 2003). Despite the links found

between memory encoding and theta activity from scalp recorded EEG, it is unclear

whether scalp recorded theta activity should be attributed to hippocampus (Andersen,

Morris, Amaral, Bliss, & OKeefe, 2006). Additionally, it is also possible that scalp-

recorded theta represents a form of neocortical theta that does not correspond to

hippocampal theta *per se*.

Although theta has garnered considerable attention in the fields of spatial and

memory research, other frequency bandwidth activity has also been associated with task

performance. Most often, alpha activity is associated with attention (Herrmann & Knight,

2001). Within parietal lobe, alpha event-related synchronization represents inhibition

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whereas alpha event-related desynchronization represents release from inhibition (Klimesch, 2012). Additional frequencies have also been linked to behavioural task performance. For example, intracranial recordings have revealed that gamma oscillations in EC and CA1 are increased during successful memory encoding (Mormann et al., 2005). Interregional coherence in gamma and theta bands has also been implicated in working memory function (Jones & Wilson, 2005).

Cross frequency modulation of medial temporal lobe theta by frontal and parietal alpha activation likely contributes to the quality of spatial information encoding as well as the ease of retrieval. When presented with sensory stimuli, theta and gamma amplitude increase whereas alpha and beta amplitude decrease (Hanslmayr & Staudigl, 2014). Further, gamma power aligns to the opposite theta phase during performance of a match-mismatch task (Hanslmayr & Staudigl, 2014). Gamma oscillation is considerably faster than that of theta, which has led some to hypothesize that the stimuli encountered by cortex are condensed into a unitary representation within a slower theta cycle in medial temporal lobe (Nyhus & Curran, 2010).

Object-Location (OL) Memory & Spatial Navigation

Arguably, one of the most important aspects of navigation is the ability to recognize a location. In addition, the ability to triangulate one's position within the framework of the environment is also critically important. One method of controlling the salience of multiple locations within a complex environment is to ensure that a particular event occurs at a specific location. For example, the appearance of a target object or cue in a particular location is likely to increase the salience of said location and its memorability.

This type of memory has been well characterized and is known as object-location (OL) memory.

The most well-known memory task of this type may be the Eals and Silverman Object Array task (Eals & Silverman, 1994). This task involves the presentation of an array of objects, statically presented on a screen or sheet of paper. Subjects study the array for some time and are then shown a second array wherein some objects have been exchanged or relocated. An appreciable sex difference favoring women has been observed on these types of object array tasks (James & Kimura, 1997). However, it is unclear whether the data gleaned from tabletop or virtual tasks translates into real world knowledge.

The relative ease with which object-location pairings can be manipulated provides an excellent opportunity for experimental manipulation of spatial information. *Here, we hypothesize that differences in OL memory during encoding and retrieval are present and measurable in behaviour. Further, these differences should be detectable via EEG.* Related to this, we have a few key predictions or hypotheses that we are able to test as a result of our paradigm. First, sex differences in spatial task performance including OL memory have been reported. More specifically, men attend to global cues whereas women tend to attend to local cues or the use of immediate landmarks (Saucier et al., 2002). Thus we predict that men and women will respond differently to manipulations of the strategy that must be used to solve the task as well as OL pairs along these cue dimensions. That said, both sexes should remember the original locations equally well when allowed to implement the strategy of their choice due to differing amounts of experience with the use of a particular strategies as well as underlying neuroanatomical differences that may contribute to reported differences. Additionally, circulating levels of

hormones have been reported to alter task performance, so it is possible that biological

circumstances prime the organism to better perform certain behaviours. We also predict

differences in how subjects respond to real, altered, and novel OL pairs. Further, it is

possible that these differences are misrepresented in tasks that do not require real

movement through space (e.g., peripersonal or table top versions of spatial tasks), so the

inclusion of a real world spatial task was also conducted.

Chapter Two

Real World Morris Task

The Morris water task (MWT) was developed to assess the ability of rodents to find an unmarked location using information derived from the environment. When searching for a hidden target, rodents must learn to rely environmental cues to successfully locate the platform (Sutherland & Dyck, 1984). In rodents, these cues tend to be distributed around the perimeter of the testing room. Rodents use a combination of somatosensory stimuli (movement, heading direction, etc.) and the geometry of the room/cues to successfully solve the task (Arleo & Rondi-Reig, 2007). The strategies and cues used to solve the task can be manipulated by the experimenter to target specific abilities or neural systems (McDonald & White, 1994). The ability to alter task demands can also create situations where the performance of a particular group can be enhanced or diminished (Roof & Stein, 1999). Saucier and colleagues (2002) showed a similar effect in humans by enforcing the use of a particular strategy through the nature of the instructions.

Most often, when sex differences are examined using the MWT in rodents, a male advantage is reported (Jonasson, 2005). Performance has been linked to differences in the volume of the hippocampus, which tends to be larger in males, as well as variations in circulating hormone levels. Korol and colleagues (2004) showed that female rats are more likely to implement an allocentric strategy during proestrus and an egocentric strategy during estrus. Increased testosterone in male rats also improves spatial task performance – though not for all spatial tasks (Spritzer et al., 2011).

Most studies of sex differences in human navigational ability rely on the use of virtual tasks that do not incorporate real, locomotion (Driscoll et al., 2005) or are tabletop/pencil and paper versions of tasks that are restricted to movement in peripersonal

space (Koppen et al., 2013). Thus, the present experiment was designed to test the hypothesis that *sex differences observed for tasks that require navigation in peripersonal space will generalize to a task that requires whole body movements through space.*

Methods and Results

Experiment 1: Methods

Subjects. Thirty-five subjects (17 males) met inclusion criteria and completed the task. Male and female subjects of similar age ($20.8\pm .46/21.4\pm .40$; $p = .32$) participated for partial course credit. No between group differences were noted for self reported mood prior to or after participating, navigational skill, or likelihood of becoming lost.

Behavioural Task Description

   Procedure. All subjects were read an identical script (appendix I) explaining the task. Following instructions, a second experimenter led subjects to the starting location for the first trial and task performance started. Subjects also completed a post-performance questionnaire. The entire experiment was videotaped by two cameras set up at 90 angles to each other with the entire task area within view. Time, strategy, and video footage were recorded for each trial. Subjects were analyzed for strategy selection per trial, from a total of eight possible strategy mechanisms that subjects could implement during each trial from recorded video footage by two trained research assistants. The eight strategies used to analyze the subjects were chaining, random, geometric, thigmotaxic, variable, direct and targeted search (for overview see Illouz et al., 2016) The University of Lethbridge human research ethics committee approved all procedures.
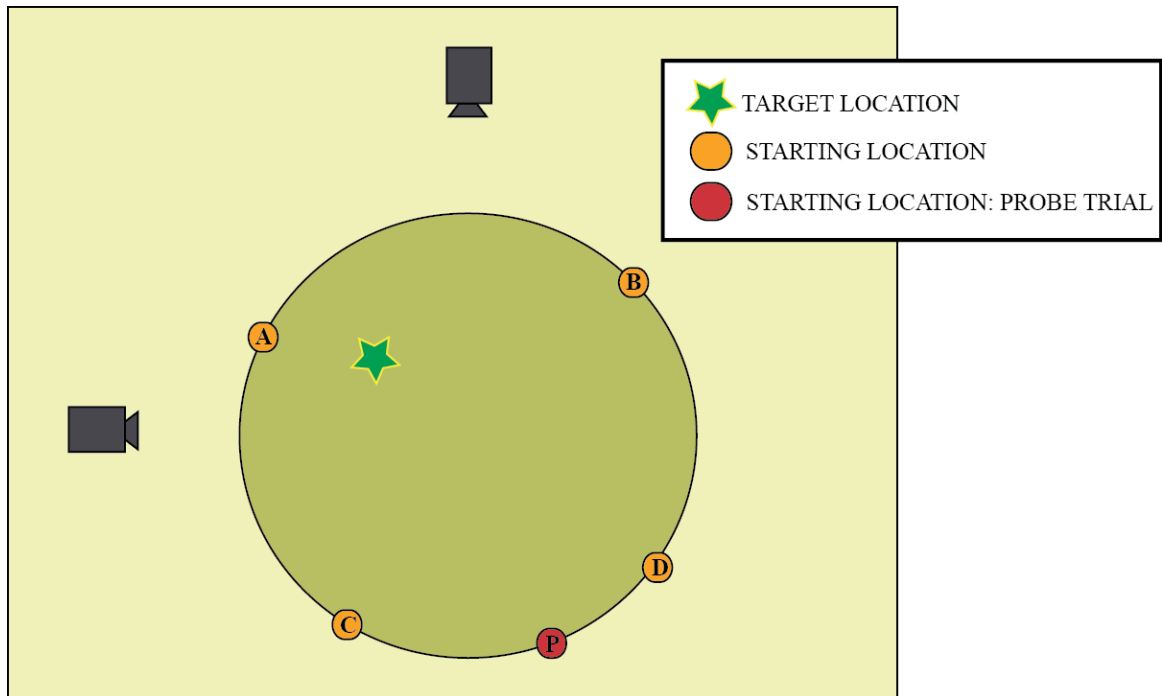
Figure 2.1. Depiction of the task area. The dark green circle represents the navigable area of the task. The orange circles represent the starting locations for each trial. The red circle represents the starting location on the probe trial. The green star represents the goal location for each trial.

Training. Subjects traversed a circular, outdoor area approximately 20 meters in diameter (Figure 2.1). Four distinct starting locations were used for all trials (order: C-D-A-B-D-A-C-B-A-B). A consistent, unmarked target location served as the target for all subjects. A ten-trial maximum was implemented for all subjects. Training was considered complete when a subject found the target location within 10 seconds on three consecutive trials or when ten trials were completed. Each trial began and ended with an auditory cue. Discovery of the target location was signalled to the subject using an auditory cue (a pleasant whistle blast from the experimenter).

Retention Test. A single probe trial was conducted immediately after task training. A novel start location was used for the probe trial. The probe trials lasted for 30 seconds regardless of whether the subject reached the correct target location.

Experiment 1: Results

Acquisition. A univariate ANOVA comparing males and females for the total number of trials to reach criterion revealed a trend favouring males ($F(1, 33) = 3.17$, $p = .08$). A repeated measures ANOVA (sex*trial) corrected (Greenhouse-Geisser) for violation of sphericity comparing the trial duration on the first four trials revealed a significant effect of trial ($F(1.2, 37.97) = 39.3$, $p < .01$), but not sex ($F(1, 32) = .04$, $p = .87$).

Retention. Performance on the probe trial was compared across sex. A one way ANOVA revealed a significant male advantage for the time spent in the target quadrant ($F(1, 33) = 7.4$, $p = .01$). Males also spent less time in the quadrant that contained the starting location ($F(1, 33) = 7.9$, $p < .01$). A trend favouring males was observed for the number of steps taken over the course of the probe trial ($F(1, 33) = 3.7$, $p = .07$). A chi square test revealed a difference in the strategy selected during the probe trial ($X(2) = 6.9$, $p = .03$), but there were no differences in how male and female subjects oriented themselves during the probe trial ($X(3) = 2.04$, $p = .56$).

Summary. Males tended to learn the task more quickly and spent more time in the correct target quadrant during the probe trial. Males were also more likely to use a search strategy that was informed by allocentric information or the geometry of the space as indicated by their likelihood to orient themselves relative to distal landmarks, whereas females appeared to orient themselves according to egocentric information.

Experiment 2: Methods

Subjects. Forty-seven subjects (24 male) met inclusion criteria and completed the task. Male and female subjects were matched for age ($21.2 \pm .37 / 20.3 \pm .33$; $p = .09$).

Females were more likely to report having poor navigational skills and increased likelihoods of becoming lost when visiting a new place (ps. < .01). No between group differences were noted for mood prior to or after participating.

Procedure. All procedures were identical to those followed in experiment one with the exception of the starting location on the second trial. Four distinct starting locations were used for all trials (order: C-C-D-A-B-D-A-C-B-A). A consistent, unmarked target location served as the target for all subjects. A 10 trial maximum was implemented for all subjects. Training was considered complete when a subject found the target location within 10 seconds on three consecutive trials or ten trials were completed. Each trial began and ended with an auditory cue. Discovery of the target location was signalled to the subject using an auditory cue (whistle blast from the experimenter).

Retention Test. A single probe trial was performed immediately after task performance. A novel start location was used for the probe trial. The probe trials lasted for 30 seconds regardless of whether the subject reached the correct target location.

<div align="center">Experiment 2: Results</div>

Acquisition. A univariate ANOVA comparing males and females for the total number of trials to reach criterion revealed significant male advantage ($F(1, 45) = 8.4, p < .01$). A repeated measures ANOVA (sex*trial) corrected (Greenhouse-Geisser) for violation of sphericity comparing the trial duration on the first four trials revealed a significant effect of trial ($F(1.1, 48.7) = 91.8, p < .01$), and sex ($1.1, 48.7) = 5.2, p = .02$). Post hoc independent samples t-tests revealed that males and females differed on the first ($T(45) = 2.51, p = .02$) and third trials only ($T(45) = 2.40, p = .02$).

Retention. Performance on the probe trial was compared across sex. A one way ANOVA for the time spent in the target quadrant did not reveal significant differences

($F_{(1, 43)} = 1.1$, $p = .24$). A chi square test revealed no difference in the strategy selected during the probe trial ($X(1) = .93$, $p = .33$), but there were differences in how male and female subjects oriented themselves during the probe trial ($X(1) = 2.0$, $p = .16$).

Summary. Males learned the task more quickly and spent more time in the correct target quadrant during the probe trial. As in experiment 1, males and females implemented search strategies that used spatial geometry and egocentric position in space, respectively.

Experiment 3: Methods

Subjects. Thirty-three subjects (13 males) met inclusion criteria and completed the task. Male and female subjects were matched for age ($21.4\pm .63/20.4\pm .36$; $p = .13$). No between group differences were noted for self reported mood prior to or after participating, navigational skill, or likelihood of becoming lost.

Procedure. All procedures were identical to those followed in experiment one with the exception of the starting location on the second trial. Four distinct starting locations were used for all trials (order: C-D-A-B-D-A-C-B-A-B). The unmarked target location moved according to the egocentric starting position for each trial (see Figure 2). A 10 trial maximum was implemented for all subjects. Training was considered complete when a subject found the target location within 10 seconds on three consecutive trials or ten trials were completed. Each trial began and ended with an auditory cue. Discovery of the target location was signalled to the subject using an auditory cue (whistle blast from the experimenter).
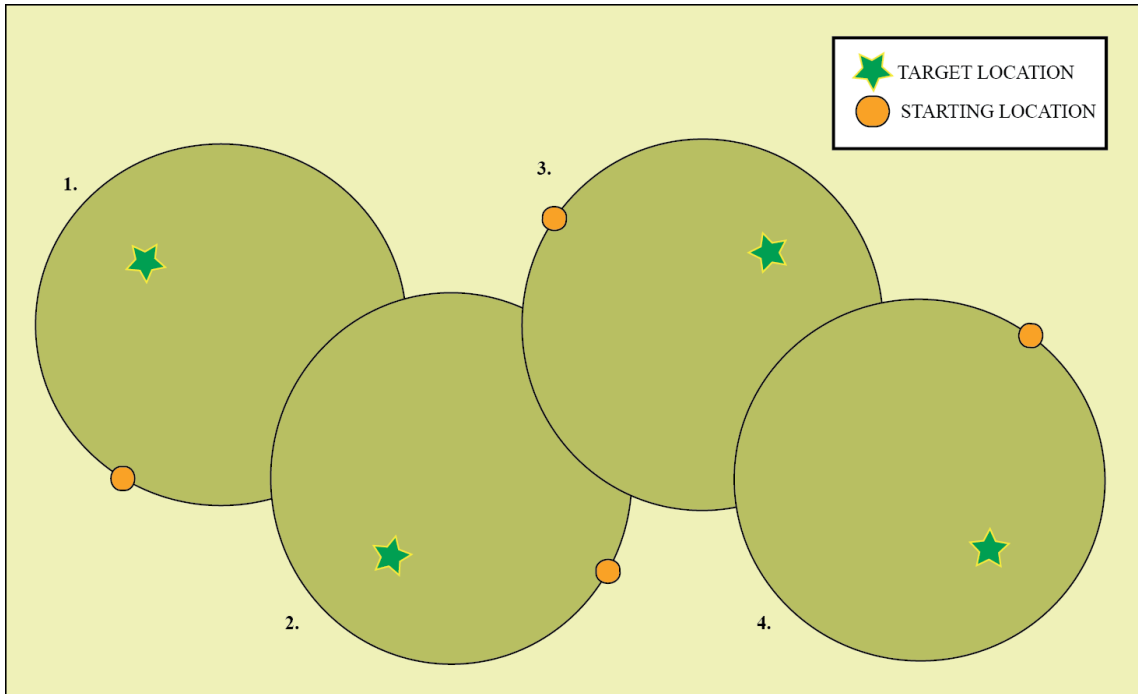
Figure 2.2. Depiction of the task area for the egocentric version of the task. The dark green circle represents the navigable area of the task. The orange circles represent the starting locations for each trial. The red circle represents the starting location on the probe trial. The green star represents the goal location for each trial. Note that the goal location moves on each trial relative to the starting location.

Retention Test. A single probe trial was performed immediately after task performance. A novel start location was used for the probe trial. The probe trials lasted for 30 seconds regardless of whether the subject reached the correct target location.

Experiment 2 summary. Males learned the task more quickly and spent more time in the correct target quadrant during the probe trial and implemented a different search strategy.

Experiment 3: Results

Acquisition. A univariate ANOVA comparing males and females for the total number of trials to reach criterion revealed a significant female advantage ($F(1, 31) = 5.3$, $p = .03$). A repeated measures ANOVA (sex*trial) corrected (Greenhouse-Geisser) for

violation of sphericity comparing the trial duration on the first four trials revealed a significant effect of trial ($F(1.6, 49.0) = 12.2, p < .01$), but not sex ($F(1.6, 49.0) = 1.0, p = .39$).

Retention. Performance on the probe trial was compared across sex. A repeated measures ANOVA comparing the time spent in the correct target quadrant according to the egocentric start position, the last found correct quadrant, and all other quadrants revealed a significant effect of quadrant ($F(2,14) = 10.5, p < .01$) and a trend for quadrant * sex interaction ($F(2,14) = 2.8, p = .09$). Chi square tests revealed a significant difference in the strategy selected during the probe trial ($X(5) = 15.8, p < .01$) as well as a difference in how male and female subjects oriented themselves during the probe trial ($X(3) = 10.52, p = .02$).

Summary. Unlike the other two experiments, a female advantage was observed for the rate of task acquisition. Further, a sex * quadrant interaction was observed whereby females spent more time in the quadrant that should house the target given the starting location. Consistent with the first and second experiments, male subjects relies on environmental cues to develop their strategies whereas females seemed to focus on egocentric information. In addition, females oriented themselves more accurately than males during the probe trial.

Collapsed Results

Subjects. The data from 113 subjects (51 males) who met inclusion criteria and completed the task were collapsed for cross-experiment analysis. No between group differences were noted for age, self reported mood prior to or after participating, navigational skill, or likelihood of becoming lost.

Table 2.1. Sample sizes for each group

Sex	Exp 1	Exp 2	Exp 3	Total
Female	18	23	20	61
Male	17	24	13	44
	35	47	33	105

Acquisition. A univariate ANOVA comparing groups for the total number of trials to reach criterion revealed significant effect of group ($F(5, 107) = 4.3, p < .01$). Post hoc comparisons were conducted using the Games-Howell test and revealed a significant difference between females in the first experiment and males in the second ($p = .004$), a difference trend between males and females in the second experiment ($p = .06$), and a significant difference between males in the second and third experiments ($p = .005$). A repeated measures ANOVA (sex*trial) corrected (Greenhouse-Geisser) for violation of sphericity comparing the trial duration on the first four trials revealed a significant effect of trial ($F(1.3, 136.2) = 113.6, p < .01$), and a trial*group interaction ($F(6.4, 136.2) = 2.6, p = .02$). *Post hoc* comparisons were conducted using the Games-Howell test and revealed a significant difference between the third experiment females and males and all other groups excluding each other ($p < .02$).
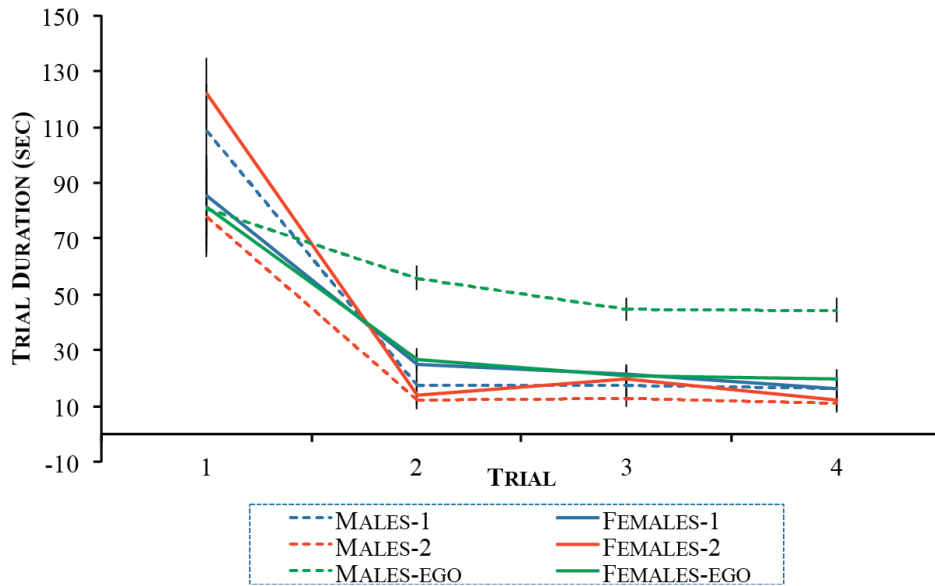
Figure 2.3. Task acquisition (all groups). Males and females -1 and -2 refer to the first and second experiment subjects. Males and females -ego refers to subjects from the third experiment.
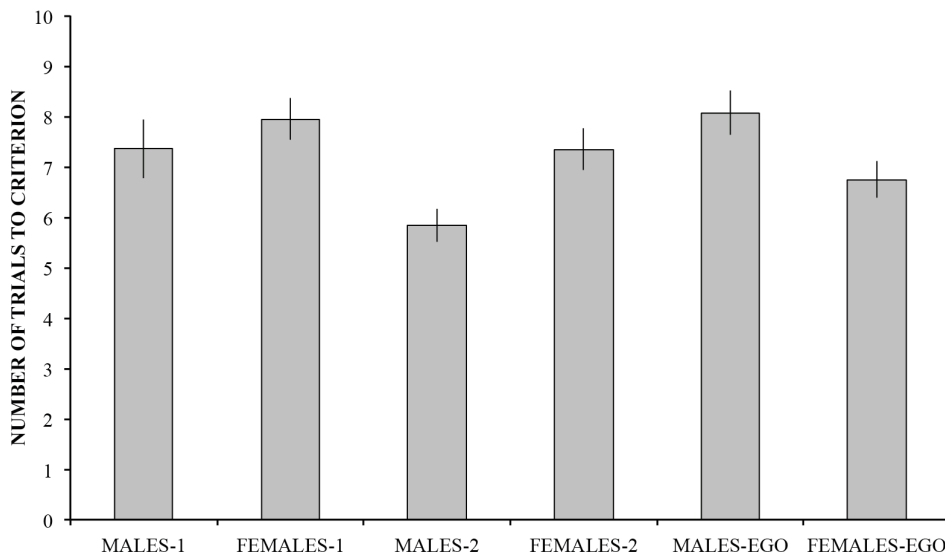
Figure 2.4. The number of trials required for task acquisition (all groups). Males and females -1 and -2 refer to the first and second experiment subjects. Males and females - ego refers to subjects from the third experiment.

Retention. A univariate ANOVA revealed a significant effect of group for the total number of steps taken during the probe trial ($F(5, 107) = 11.2, p < .01$). *Post hoc comparisons were conducted using the Games-Howell test and revealed a significant*

difference between the males and females in the second experiment ($p = .05$) and between males in second and third experiments ($p < .01$). Chi square tests revealed significant group differences on the strategy selected during the probe trial ($X(25) = 75.1, p < .01$) as well as a difference in how male and female subjects oriented themselves during the probe trial ($X(3) = 156.4, p < .01$).
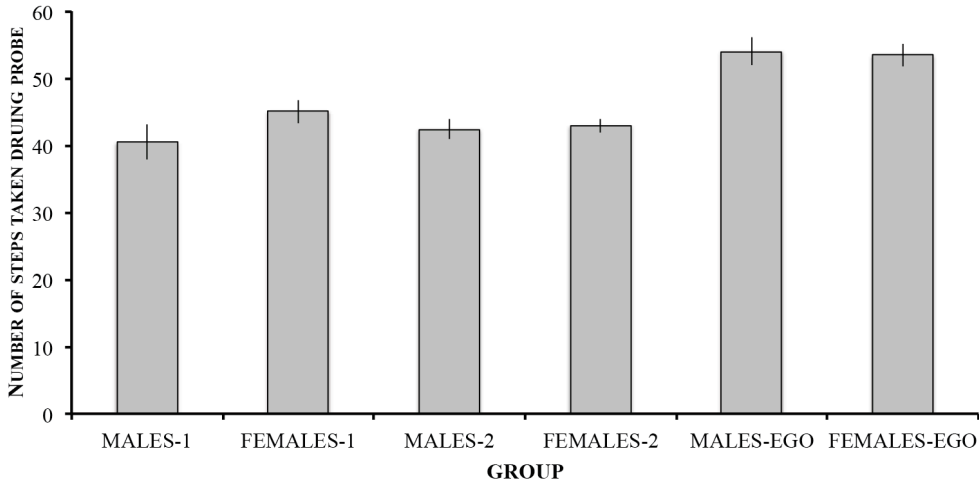
Figure 2.5. Number of steps taken during the probe trial. Both males and females in the egocentric condition took more steps than all other groups.
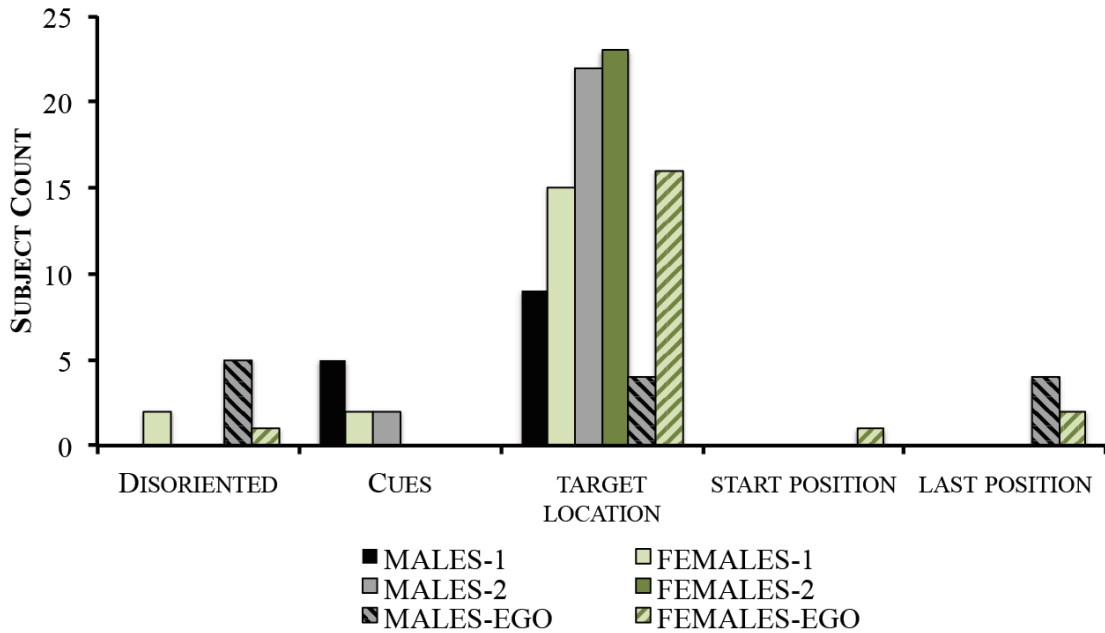
Figure 2.6. Initial orientation during the probe trial for all groups.
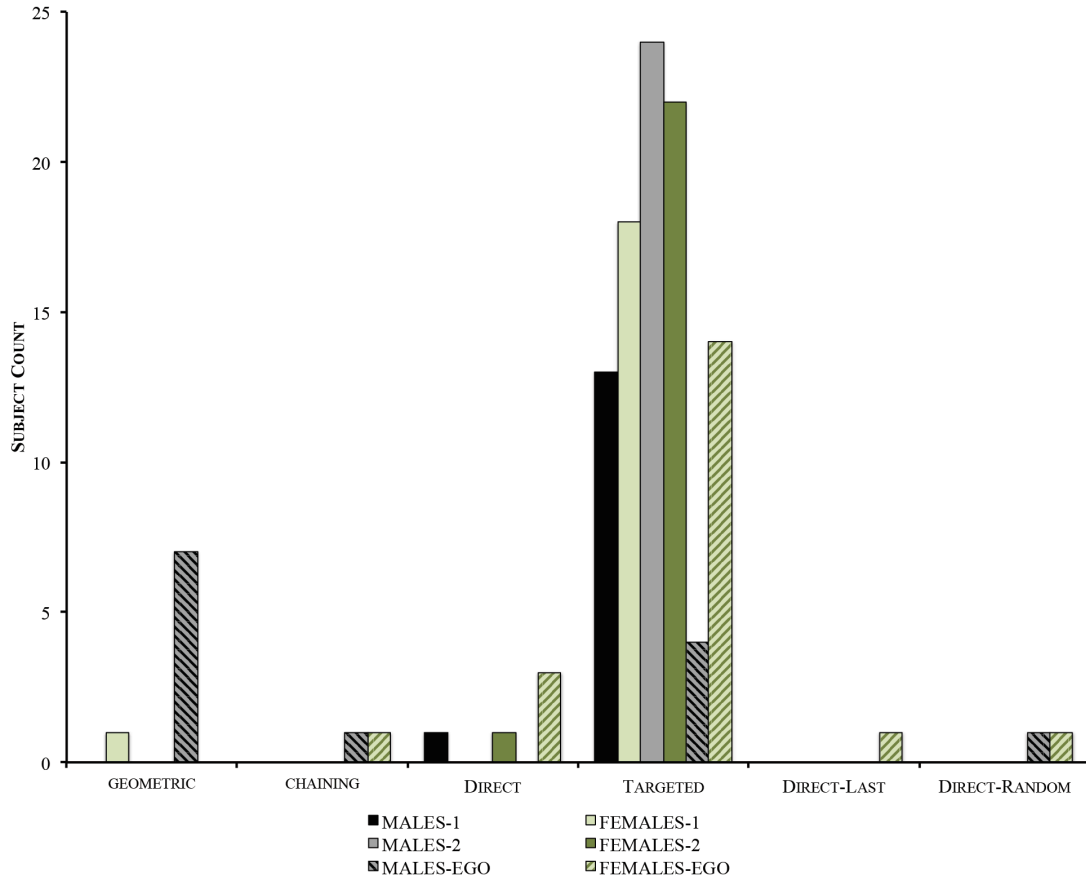
Figure 2.7. Strategy selection during the probe trial for all groups.

Discussion

A male advantage was observed for the first and second experiments wherein strategy selection is freely chosen and the subjects are able to solve the task according to self-determined strategies. However, when subjects were forced to rely on an egocentric strategy, a female advantage emerged. That said, one could argue that there is no real female advantage. Rather, there is a male disadvantage when subjects are forced to rely on an egocentric strategy because females in the third experiment show similar rates of acquisition to females and males in the other two experiments whereas male performance is worse. These results were consistent with reports that males and females seem to have

different default strategies during the performance of spatially demanding tasks (Saucier et al., 2002). Further, performance on this task is consistent with behavioural patterns observed during performance of virtual navigation tasks providing justification for the continued use of virtual tasks for assessing spatial abilities in human subjects (Driscoll et al., 2005).

The results of this study are novel because we have managed to structure our task in such a way that the influence of strategy can be examined. Holding constant the target location relative to the starting position, requires subjects to use egocentric information to solve the task. When subjects are forced to do so, there is little change in the performance of female subjects whereas the performance of male subjects who are forced to rely on an egocentric strategy is considerably worse than all other groups. This finding also implies that females may be using an egocentric strategy to solve tasks when they are able to determine their strategy independently. The enhanced performance amongst the male subjects in the first and second studies relative to females may be a reflection of the accuracy of the strategies that are being implemented to solve the task rather than superior male performance.

Egocentric strategies may rely on attention to contextual information that is processed in LEC although the process of path integration – which can be argued to also be egocentric in nature, is processed in MEC. Regardless, the spatial representations supported by LEC lack the allocentric spatial accuracy that is found in MEC because of the nature of the information that is processed in these two distinct subregions (Knierim & Neunuebel, 2015). Conversely, the nature of the geometric information that is processed in MEC imply that reliance on this system would lead to increased accuracy during the performance of spatially demanding tasks because more information about

position in space is contained in the outputs from MEC. As a result, geometric and allocentric strategies are more likely to result in increased accuracy of performance. This prediction is confirmed by what we have observed in the performance of the real world task.

It is not enhanced spatial ability amongst male subjects *per se* that is reflected in their improved performance of spatial tasks, rather it is improved accuracy associated with the implementation of allocentric strategies relative to egocentric. The reason why males and females differentially implement these strategies is still not understood, but alterations to the default strategy implemented by female subjects across the estrus cycle imply that this difference may be mediated by hormones (Korol et al., 2004). The existence of sex differences is unequivocal, the underlying causes of these difference is not.

Virtual Task Procedures and Design

The results of the first series of experiments provide justification for the continued use of virtual tasks to approximate navigation through the real world. In addition, depth electrode recordings in human epilepsy patients have shown that the activity of neural regions active during navigation in nonhuman animals is very similar in human subjects during the performance of virtual navigation (Ekstrom et al., 2005; Ekstrom et al., 2003; Lega et al., 2012). Whereas place cells are often observed in the hippocampi of rodents, Georges-François, Rolls, and Robertson (1999); Rolls (1999) have reported spatial view cells in monkey hippocampus that may be analogous to rodent place cells as well as place cells recorded using implanted intracranial recordings conducted in humans during virtual movement (Niediek & Bain, 2014).

A considerable body of literature has amassed on virtual navigation tasks. Virtual tasks tend to be set in either naturalistic/outdoor settings, recreations of Morris water tasks setups in nonhuman, animals building interiors, or city streets (Astur, et al., 1998; Burgess et al., 2002; Maguire et al., 2000). Each task type has advantages and provides insight into how humans represent different types of spaces. The use of naturalistic environments provides an opportunity to study unrestrained movement (i.e., an environment where decision points are self-determined relative to reaching intersections in interior or urban settings). A virtual environment was developed in order to further assess the influence of environmental and task factors on spatial task performance. A male advantage is often reported on virtual versions of the Morris Water task whereas a female advantage is often reported for tasks assessing recognition of the rearrangement of objects/locations. Thus, this virtual navigation paradigm is a combination of both types of

knowledge and various manipulations thereof in order to begin to deconstruct what underlies these propensities.

General Methods

The University of Lethbridge human research ethics committee approved all procedures.

Behavioural Task

   User Interface. All stimuli were presented to the subject on a 24.1-inch NEC 242W-BK monitor (average response time 8 ms) run on Dell Mobile Precision M6600 laptop. Subjects were seated 57cm from the monitor in a wooden chair. Subjects were asked to keep their feet flat on the floor and to find a seated position they could maintain with minimal movement for approximately one hour. Subjects moved through the virtual environment with a joystick that had been adapted using an Arduino® kit in order to facilitate the ability to send event triggers to the EEG data acquisition system. Subjects could toggle between running (fast) and walking (slow) by modifying the degree of forward tilt on the joystick. Subjects were not able to travel in the reverse direction and speed was constrained to fast, slow, or still. Subjects were provided with a wireless mouse (Logitech ®) for the topographical translation phase of the task.

   Virtual Environment. A virtual environment with a 60° field of view was created in UDK (Epic Games, Gary, NC, USA) and resembled a large, rolling plain bordered by mountains, water, and cliffs (Figure 3.1a-d). The subject was constrained inside a navigable area by a white fence, but the scenery beyond the fence was visible (see Figure 3d). The navigable area contained several salient environmental features (e.g., rock formations, tree stands) as well as three obelisks that differed in size (height and thickness) and color that served as landmarks. Distal landmarks included a mountain

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range with various faces and peaks, hills, and a seascape. Task performance occurred in three parts: Acquisition, memory retention, and a topographic translation test.
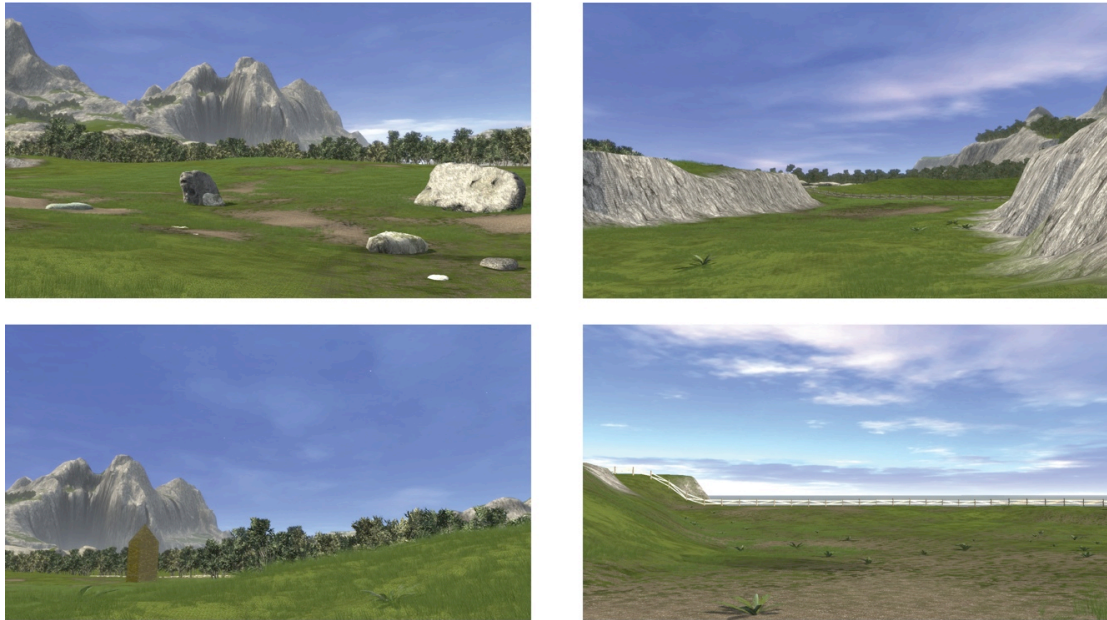
Figure 3.1 (a-d). Screenshots of the virtual environment. Target objects appeared when subjects were near them, but were otherwise invisible to the subject.

Exploration Phase. Subjects were asked to freely explore the environment via a joystick. The acquisition phase required subjects to locate 8 target objects that were distributed throughout the navigable area of the environment (Figure 3.2). The distribution of target objects was such that one could be found inside of each eighth of the environment. Acquisition ended when the eighth target object was located, but subjects were told that the first phase lasted a random amount of time between 15 and 20 minutes. The density of proximal cues (e.g., rocks, tree stands) scattered throughout the navigable space was controlled to ensure that half of the targets possessed a high cue density and half were paired with a low proximal cue density. Target objects were not visible until the subject was approximately 5 virtual meters from them. Target objects appeared in the same orientation regardless of angle of approach.
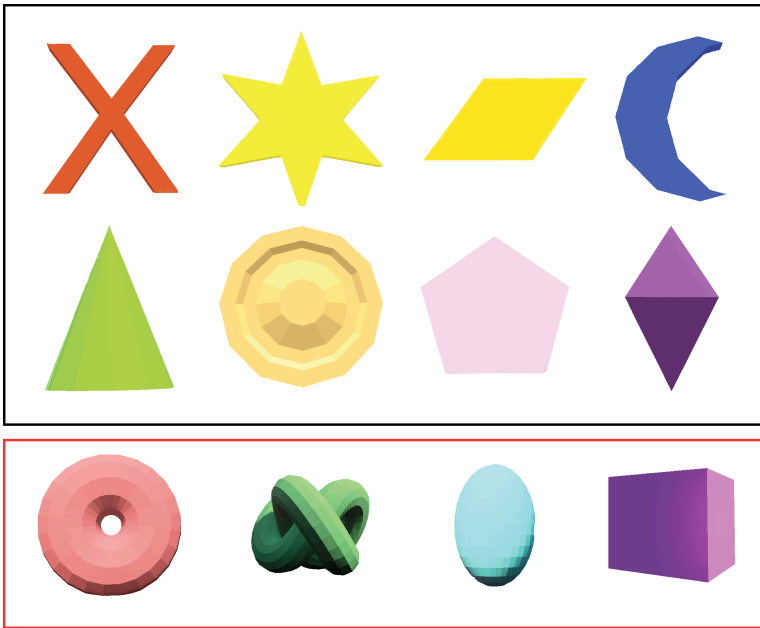
Figure 3.2. Target objects. Objects located inside the black box represent objects encountered as the subjects explored the virtual environment. The objects located in the red box represent foil objects that were not encountered in the virtual environment.

Subject controlled movement via joystick (ends trial with button press at desired location.
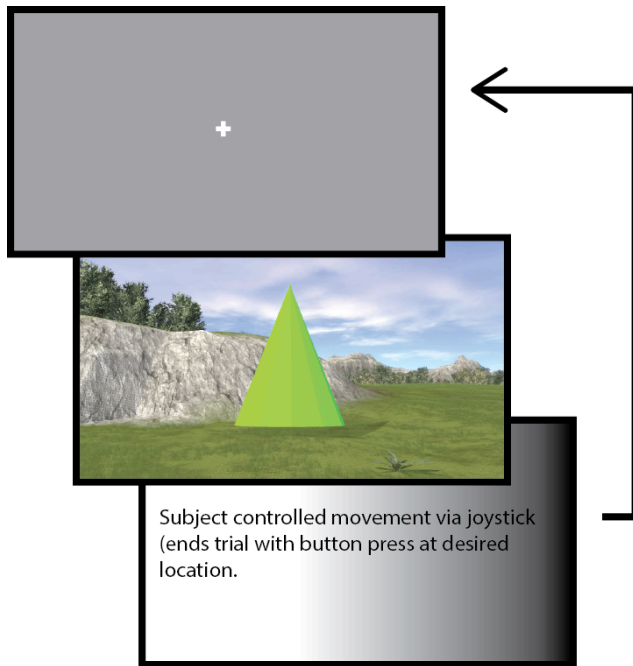
Figure 3.3. Trials Sequence. Each trial began with a fixation cross followed by a target comprised of a background an object (presented simultaneously). The subject then appeared in the centre of the environment and controlled their movement to their desired target.

Target Trial Probes. For the memory phase of the task, subjects were shown a fixation cross, followed by a screenshot that included a target object and visible background corresponding to a location experienced during the acquisition phase of the task (Figure 3.3). After the presentation of the stimulus, the subject appeared in the centre of the navigable environment, navigated to their target, and pressed a trigger to and that trial. Prior to initiating movement on each trial, subjects were asked to rotate until they were facing what they considered the optimal direction and then travel in as straight a line and as quickly as possible to the target for that trial. Once subjects reached their chosen location, they pressed a trigger on the joystick that initiated the next trial. A crosshair was presented on the screen to ensure the subject fixated on the centre of the screen for 0.5 - 0.75 seconds followed by the static presentation of a target object in the environment. The target remained on the screen for 3 seconds. Subjects began each trial facing the same direction in the centre of the navigable area. Subjects were asked to rotate until they felt they were optimally oriented toward the target object. Subjects traveled to where they thought they had encountered the object and pressed a trigger on the joystick thereby ending the current trial and continuing to the next trial. Target objects never appeared during this phase of the task so subjects never received feedback on target accuracy.

Of the 100 trials, 40 were true trials wherein the target object and background location were congruent with what the subject had encountered during the exploration phase of the task, 40 were incongruent or mismatch trials wherein target objects were placed in locations that had been paired with a different object during exploration, and 20 foil trials wherein new target objects were presented in locations outside the navigable area (Figure 3.4). The first 24 trials were all true trials (e.g., object A-location A) to eliminate interference effects.

Figure 3.4. Sequencing of 100 trials during the retention phase of the task. Green represents true/congruent trials, yellow represents mismatch/incongruent trials, and red represents foil trials.

Topographic Translation Task. A drag-drop program developed in Adobe Flash was incorporated in order to evaluate how subjects would translate their navigation experience into a topographical representation of the explored virtual environment (Figure 3.5). Using a mouse, subjects were asked to click on and drag pictures of the all target objects to where they thought they belonged in the environment.
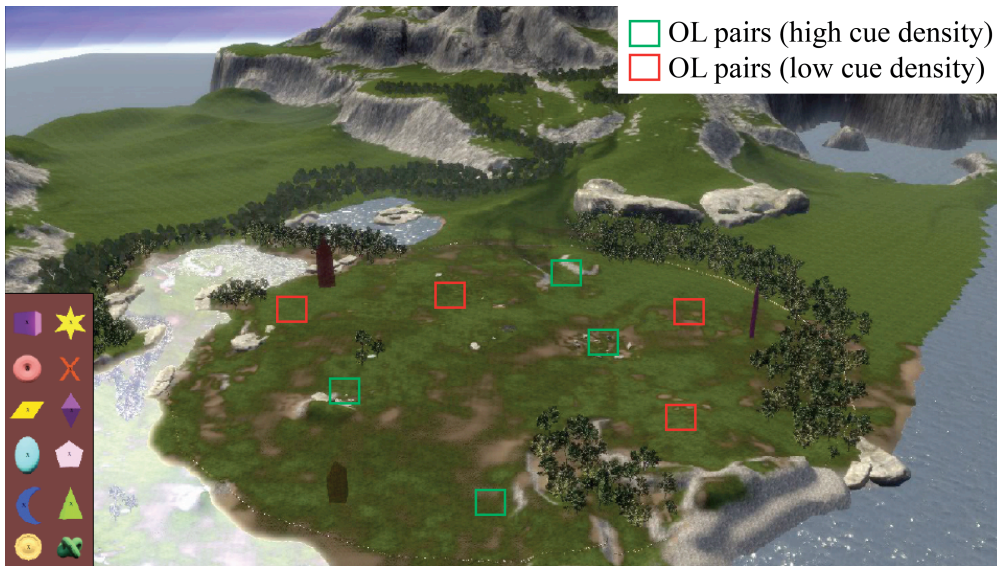
Figure 3.5. Topographical translation task image. Subjects were presented with this image and a block of movable objects along the left hand side of the screen. Using a mouse, subjects would drag the target objects to their desired location. Subjects informed the experimenter when they were satisfied with the placement of all objects and the experiment ended. The green boxes in the figure refer to the objects that had a high promixal cue density. The red boxes correspond to objects with a low proximal cue density.

Physiological Measures

Electroencephalography. The electroencephalogram (EEG) was recorded at 500 Hz using a 128 Ag/AgCl electrode geodesic net (Electrical Geodesics Inc., Eugene, OR). The montage was initially referenced to the vertex and then digitally re-referenced to an average reference. Data were imported into the BESA software package (Megis Software, Grafelfing, Germany) for further analysis. Impedances were maintained below 100 kΩ. Data were visually inspected for bad channels (e.g., movement artifacts) and these were interpolated *ad hoc*. If more than 15 channels (approximately 15% of total electrode number) required interpolation, data for that subject were discarded. Ocular artifacts were corrected using an adaptive artifact correction algorithm (Ille et al., 2002). HEOG and VEOG threshold voltages were 150 μV and 250 ΩV respectively. Data were high- (0.6 Hz, 6 dB/octave) and low-pass filtered (30 Hz, 12 dB/octave) with zero-phase Butterworth filters and were transformed into a standard 10-20 electrode montage.

Time-frequency analysis (Hoechstetter et al., 2004). Time frequency (TF) plots (range: 2 – 30.0 Hz; 2.0Hz/25ms sampling resolution) were created for 1500 ms epochs that began 500 ms before stimulus presentation. This allowed for a baseline taken from the prestimulus interval while subjects were supposed to be fixating on the fixation cross. TF plots from individual subjects were collapsed according to the relevant hypothesis and total, evoked, and induced power were assessed. TF plots were created with the Fieldtrip (F.C. Donders Centre, Nijmegen, Netherlands) and EEGLAB toolboxes for Matlab.

Hormone Analysis. Testosterone and estradiol were assayed from saliva using enzyme immunoassay kits (ELISA, Salimetrics Inc., Carlsbad, CA). After signing consent forms, subjects were asked to gently rinse their mouth using bottled water. A 2 mL passive drool saliva sample was collected after a 10-minute waiting period. Samples

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were divided into two storage tubes and stored at -80° until processed. Testosterone and estradiol assays were conducted on samples collected from all subjects to examine the relationship between task performance, strategy selection and circulating gonadal steroid levels. All assays were run in duplicate.

Binding specificity and inter-well reliability: estradiol. Specificity of the antibody used for the estradiol assay exhibits cross reactivity (%) for estriol (0.234), estrone (1.276), prednisone (0.016), and ethynylestradiol (0.189). The average coefficient of variability (CV) for the interwell variability is .099 CV.

Binding specificity and inter-well reliability: testosterone. Specificity of the antibody used for the testosterone assay exhibits cross reactivity (%) for androstendione (1.157), 21-deoxycortisol (0.004), dianabol (0.489), dihydrotestosterone (36.4), epitestosterone (0.165), 11-hydroxytestosterone (1.90), 19-nortestosterone (21.02), estradiol (0.025), estriol (0.012), estrone (0.005), and progesterone (0.005). The average coefficient of variability (CV) for the interwell variability is .102 CV.

Chapter Four

Sex Differences

Sex differences in spatial abilities have been reported across a range of species and are often attributed to sex-specific evolutionary selection pressures (Sherry & Hampson, 1997). Typically, males are categorized as 'better' navigators in terms of absolute target accuracy or when the task requires the use of cardinal directions. Males tend to employ this type of strategy under conditions where instructions about strategy are ambiguous whereas females tend to use landmark based strategies (Andersen et al., 2012). Conversely, superior performance by females is often observed on object location memory tasks (e.g., Object Location Memory Task)(Silverman et al., 2007). Females also exhibit a propensity to implement a landmark-based strategy in lieu of instruction (Rahman et al., 2005). These propensities appear to be preserved across mammals (Clint et al., 2012), which is consistent with the differences being at least in part an innate feature of biological sex and not entirely due to gender-related experiential factors such as differences in how we educate boys and girls about space.

Beyond these between-sex differences, hormone levels may alter performance within each sex (Andreano & Cahill, 2009; Hausmann et al., 2000). Considerable evidence has amassed outlining the organizational effects of sex hormones during early and prenatal development (Berenbaum & Beltz, 2011), but differences in navigation-task performance mediated by circulating levels of gonadal steroids have been inconsistently supported in the literature. Alterations in hippocampal neurogenesis (Pawluski et al., 2009) and hippocampal volume (Protopopescu et al., 2008) have been reported, but how these anatomical changes influence behaviour is less clearly understood. Circulating levels of gonadal steroids can influence performance on virtual spatial tasks (Driscoll et al., 2005).

Salivary concentrations of testosterone and estradiol are strongly correlated with task performance and the inclusion thereof in analyses could: (1) help elucidate the role of circulating hormone levels on hippocampal-dependent tasks; and (2) account for a proportion of the variance and enhance our ability to detect other factors of interest. Thus, the aim of the current experiment is to assess the hypothesis that *differences in target accuracy/ memory, and attended navigational cues/strategy selection will be mediated by hormonal status and biological sex.*

Methods

Subjects. Twenty-three males and 13 females (aged 20 – 25) were used for all comparisons except where otherwise noted. Student subjects were recruited from undergraduate Psychology and Neuroscience courses and were given 2% course credit for participation. All subjects had normal or corrected to normal vision and were free of psychiatric diagnoses or medical illness. Subjects were asked to fill out a brief survey before task completion.

Behavioural Task. A more detailed description of the behavioural task can be found in Chapter Two.

Statistical Analysis. Behavioural data were analyzed in SPSS (v 21).

Results

Phase I: Acquisition

Task Acquisition. A univariate ANOVA revealed no significant differences between males and females for the duration of the exploration phase of the task ($F(1, 34) = .393$, $p = .54$) nor path length, ($F(1, 34) = .121$, $p = .73$). No Spearman correlations were observed for path length, duration of exploration, or testosterone, ($r_s = .2$, ps > .05). A

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significant correlation was observed for time spent during exploration and estradiol, $r_s =$ .48, $p < .01$.

Phase II: Targeted Search

Path-length

　　True trials. A repeated measures ANOVA for average path-length to each of the target locations corrected using Greenhouse-Geisser due to sphericity violation did not differ for sex or object ($p > .05$), but a between subjects effect was observed for object ($F(1,35) = 4.57$, $p = .04$). This effect makes sense given the differences in distance from the start location to each object.

　　Incongruent Trials. A repeated measures ANOVA comparing the composite path-length on true vs. incongruent trials did not reveal any significant differences by sex ($F(1, 35) = 1.32$, $p = .26$) or navigational skill ($F(1, 35) = 0.01$, $p = .93$). The lack of effect on these measures is likely due to large variability within each condition. Because no between group differences were observed, paired t-tests were used to assess true vs. incongruent trials (see Table X).

Table 4.1. Paired samples *t*-test for path-length on true vs. incongruent trials.

Object Pair (true - incongruent)	Mean	Std. Error	t	df	Sig. (2-tailed)
Composite - All	-122088.06	87216.11	-1.40	36	0.17
Composite - High cue density	-140487.24	96039.96	-1.46	36	0.15
Composite - Low cue	-103688.88	78403.25	-1.32	36	0.19

density					
Moon	-109129.96	69520.25	-1.57	36	0.13
Parallelogram	11597.54	3735.16	3.11	36	< 0.01
X	-143964.27	104693.78	-1.38	36	0.18
Hexagon	-94223.72	69588.69	-1.35	36	0.18
Cone	-155200.51	104702.72	-1.48	36	0.15
Star	-298469.24	214307.95	-1.39	29	0.17
Diamond	-94599.68	69827.15	-1.36	36	0.18
Coin	-149513.15	104941.30	-1.43	36	0.16

Target Placement During Search.

Object Placement: True trials. A 2*8 (sex*object) repeated measures ANOVA for the RMSE for each of the eight objects (each object is encountered 5 times) revealed a significant effect of object ($F(7, 245) = 26.78$, $p < .001$) and sex ($F(7, 245) = 3.10$, $p = .004$). The inclusion of incongruent trials could interfere with the true object location pairing. Thus, a repeated measures ANOVA comparing the first three trials (wherein subjects had only experienced true OL pairs) to the last 2 pairings for each true OL (wherein the subject would have encountered incongruent trials for each object) revealed a significant effect for some objects, but no significant effects of sex were observed. Free testosterone and estradiol were not associated with object placement.

Based on the reported propensity of females to attend to local cues and males to attend to the global geometry of the space, it was predicted *a priori* that the sex difference in accuracy would be constrained to targets with a low density of cues in the immediate vicinity. Thus, objects were collapsed into low (parallelogram, X, hexagon, and diamond)

and high (moon, cone, star, coin) cue density conditions. Repeated measures ANOVA revealed a significant effect of object ($F(1, 35) = 115.87, p < .001$) and an object*sex interaction ($F(1, 35) = 7.33, p = .01$). Though sex influenced task performance, bound testosterone and estradiol were not significantly correlated with task performance ($ps. > .05$)

Incongruent Trials

Choice. A chi square test comparing choice for object placement relative to the objects original location vs. the background present on a particular trial collapsed across all objects did not reveal a significant sex difference ($X(1) = 0.95, p = .33$). However, given the observed sex differences on true trials for objects with high vs. low local cue density, chi square tests were used to compare males and females on composites of the low and high cue density objects. No significant difference was observed for the high cue density objects ($X(1) < 0.01, p = .97$), but a significant difference was observed for the low cue density objects ($X(1) = 4.68, p = .03$).

Phase III: Object Placement Task.

Placement of True Objects. A 2*8 (sex x object) repeated measures ANOVA (Greenhouse-Geisser corrected for sphericity violation) comparing the RMSE score for object placement during the topographic translation task revealed a main effect of object ($F(3.41, 98.82) = 24.69, p < .001$) and a sex*object interaction ($F(3.41, 98.82) = 3.9, p = .008$). Pairwise comparisons for sex revealed a male advantage (M±SEM = 34.63±15.50, $p = .033$). Objects were collapsed into average scores for all objects, those with a high local cue density, and those with a low local cue density. *Post hoc*, one way ANOVAs revealed a significant sex difference for the average RMSE for all objects ($F(1, 34) =$

5.71, $p = .023$) and for the average RMSE for objects with low local cue density ($F(1, 32)$ = 5.46, $p = .026$), but not for objects with a high local cue density ($F(1, 34) = 3.9$, $p = .127$).
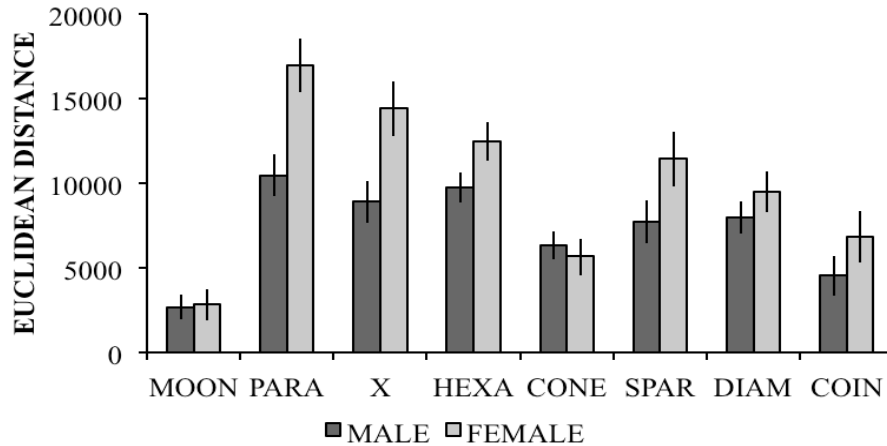
Figure 4.1. Traversal to object locations during the trials version of the task. Subjects were asked to travel directly to the target object and press a trigger on the joystick indicating they had achieved their perceived correct location and to move on to the next trial.
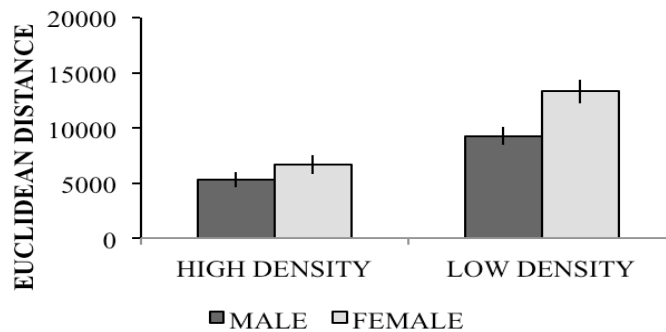
Figure 4.2. High and low density composites for error (Euclidean distance) for male and female subjects. Males perform equally well on both the high and low density conditions, whereas females perform more poorly in the low cue density condition.
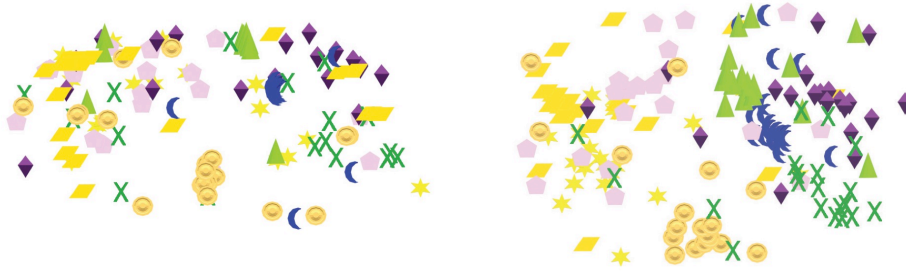
Figure 4.3a-b. Demographic representation of object placement by female (a) and male (b) subjects on the topographical translation task. Males object placement is more accurate and exhibits increased boundary extension.
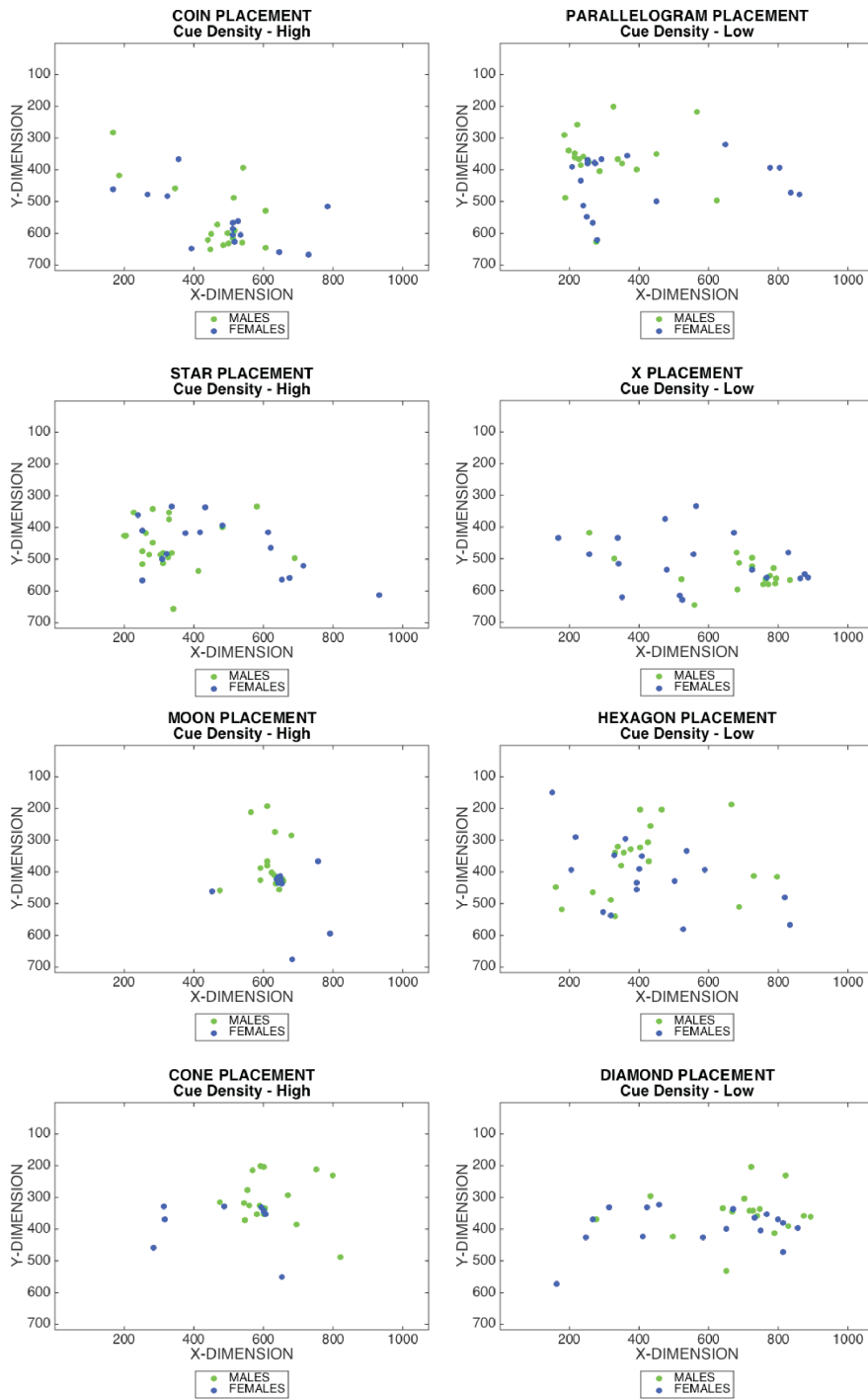
Figure 4.4. Plotted coordinates for all objects. The x and y dimensions correspond to the pixel distribution for the object placement task and males and females are plotted for each object.

Foil Trials. A chi square test comparing whether males and females showed (1) no preference or a preference for placing objects (2) inside or (3) outside the navigable area revealed a male advantage ($X(2) = 6.78$, $p = .03$) possibly indicating increased confidence or better memory or an improved capacity for making spatial inferences amongst male subjects.

This finding is corroborated using a chi square test for task performance. A median split using object placement for each object was conducted. Subjects who placed 1-4 objects below the median error distance were categorized as good navigators and subjects with 5-8 object placements above the median error distance were categorized as poor navigators. The proportion of males and females in each condition did not differ ($X(1) = .95$, $p = .33$). However, when the median split was conducted using only high ($X(1) = .001$, $p = .97$) or low density cue objects, the difference emerged for low density objects alone ($X(1) = 4.68$, $p = .03$).
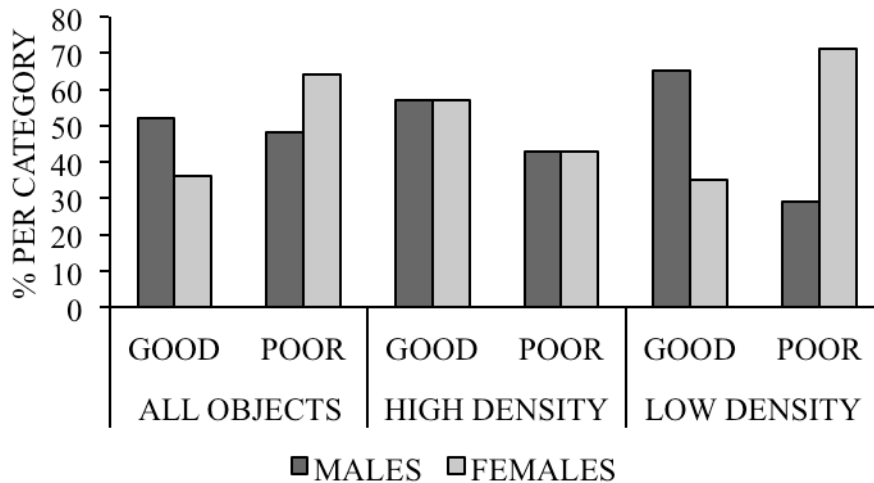
Figure 4.5. Category of object placement accuracy on the topographical translation task. As was observed for the navigation to targets, male performance was preserved under the cue sparse condition whereas good performance amongst female participants requires more local cues.

Table 4.2. Summary of virtual task performance differences in performance for male and female subjects.

Measure	Direction of Sex Difference
Duration of exploration phase	nil
Path length during exploration	nil
Path length to targets during trials	Shorter pathlength (females)
Target location accuracy	Male advantage
Location choice (original vs. incongruent)	Females choose original location most often
Object placement on topographical task	Males more accurate

Discussion

The experiment described in this chapter provided support for the hypothesis that differences in target accuracy/ memory, and navigational cues/strategy selection will be mediated by hormonal status and biological sex. When presented with ambiguous information during mismatch trials, female subjects tended to return to the original location in the cue sparse condition and to attend to the cues present in the immediate vicinity of the object, rather than use the distal cues present in the background geometry of the scene to place the target objects as the males did.

Males and females were indistinguishable during the exploration phase of the task, although there was a significant correlation between time spent during exploration and estradiol. There were differences in the path lengths to some objects, but these did not differ by sex or hormone status. Sex influenced task performance, but bound testosterone and estradiol were not significantly correlated with task performance. One of the major

differences between sexes was for the placement of objects. As predicted, there were no sex difference in the placement of objects that had a high local cue density. A male advantage emerged for the objects characterized as having a low local cue density, which was measured by having blind observers list the cues they saw when presented with the stimuli for the same length of time participants viewed the scenes. Objects that had more than 3 local cues identified by all blind observers were categorized as high cue density and objects that had fewer than 3 local cues were categorized as low cue density. A chi square test comparing whether males and females showed: (1) no preference or a preference for placing objects (2) inside or (3) outside the navigable area revealed a male advantage possibly indicating increased confidence, better memory, or an improved capacity for making spatial inferences amongst male subjects.

In Chapter 5, subjects who placed 1-4 objects below the median error distance were categorized as good navigators and subjects with 5-8 object placements above the median error distance were categorized as poor navigators. The proportion of males and females in each condition did not differ. However, when the median split was conducted using only high or low density cue objects, the difference emerged for low density objects alone wherein females performed more poorly than their male counterparts.

These differences in how males and females perform the task indicate that they are interpreting the information in different ways and may be relying on differential recruitment of neural regions to solve the task.

Chapter Five

Differences in EEG Profiles by Task Performance

The electrical activity of the brain oscillates and the frequencies and amplitudes of these oscillations are associated with particular cognitive and/or neural phenomena (Basar, 2012; Luck, 2005). In some cases, several different cognitive phenomena may correspond to activation in particular frequency bandwidths. For example, activity in the theta band (~3 - 12 Hz) is implicit in virtually all voluntary behaviour in mammals (Buzsaki, 2005). Of particular importance to the present study, oscillations in the theta and delta bands are consistently implicated in navigation and memory when localized to the PHC (Buzsaki & Moser, 2013; Ekstrom & Watrous, 2014; Serruya, Sederberg, & Kahana, 2014). In addition to localized patterns of activity, the synchronization of the activity across regions appears to be important for memory formation (Fujisawa & Buzsaki, 2011). The inclusion of different groups and comparisons between them may reveal that particular groups are more sensitive to particular manipulations than others. The best and the worst scoring subjects were collapsed into two groups in order to test the hypothesis that *the responses to congruent and incongruent OL pairs will be distinguishable between good and poor navigators in the time frequency domain. It is possible that poor navigators will exhibit increased activity relative to controls because they find the task more difficult. Conversely, it is possible that good navigators will exhibit increased power in response to stimulus presentation and that this increased reflects improved fidelity in the signal amongst the good navigators relative to the poor group.*

Methods

Subjects. Euclidean distance and path length were calculated for all true object trials for all subjects (n = 41) and the five subjects who consistently placed objects the most and the least accurately, were used to populate groups of good and bad navigators that could be compared. Groups were constructed in this way to avoid overlap between the two groups to ensure between-group independence.

Physiological Measures. EEG data were continuously collected throughout the experiment. EEG data were re-referenced/interpolated into an 81-channel 10-20 electrode montage in BESA and exported to Matlab for subsequent analysis. Electrodes Fpz, Fz, F3, and Pz were selected for analysis because they are purported to correspond to activity in neural regions associated with decision-making and error detection (Fpz, Fz, F3) as well as task-specific visual/spatial information processing (Pz).

Time-frequency analysis. Data were preprocessed in BESA 5.3 and exported for analysis in Matlab with the *Fieldtrip* toolbox. Intertrial coherence (ITC), total (TSE), evoked power (EP), and induced power (IP) across theta, alpha, and beta activity bands were adjusted by pre-stimulus baseline and trial conditions were compared using a Monte Carlo permutation test after being corrected for multiple comparisons using the false discovery rate (FDR; Benjamin and Hochberg, 2005). Time-Frequency plots of ITC, IP and EP for each trial condition as well as the differences between were created using Matlab.

Results

True

An FDR corrected Monte Carlo permutation test was performed on the time-frequency data comparing how good and poor navigators respond to the presentation of

true trials at electrodes Fpz, Fz, and F3. Differences in ITC, TSE, IP and EP between good and poor navigators did not survive FDR correction. However, increased EP, at lower frequencies was observed amonst the good navigators relative to the poor (p = 6.2057e-05, FDR corrected α = 0.05) at electrode Pz.
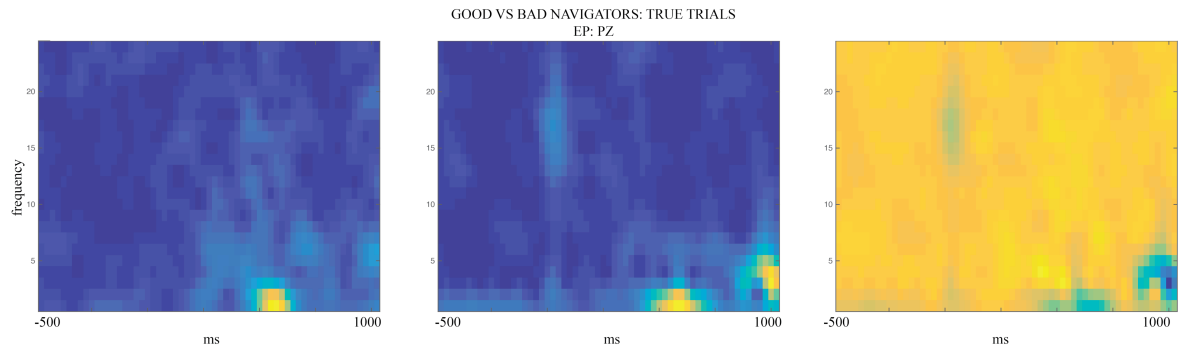
Figure 5.1(a-c). EP during true trials in good and bad navigators (a) EP @ Pz for good navigators (b) EP @ Pz for bad navigators (c) difference in EP between good and bad navigators at electrode Pz.

Incongruent

An FDR corrected Monte Carlo permutation test was performed on the time-frequency data comparing how good and poor navigators respond to the presentation of incongruent/incongruent trials at electrodes Fpz, Fz, F3, and Pz. Differences in ITC, TSE, IP and EP between good and poor navigators did not survive FDR correction. The absence of differences between the good and bad navigators were observed for the incongruent trials. There are several possible explanations for this including a lack of power, highly variable performance in both groups, the use of an innappropriate imaging technique or the complex nature of the task and its demands. It is likely that high variability in how each subject is interpreting the information contained in the stimuli as well as differences in decision making and detection of manipulations by both good and poor navigators overwhelms between-group differences.

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Discussion

The results from this chapter do not provide support for the hypothesis that differences in how good versus poor navigators respond to congruent and incongruent OL pairs will be detectable with scalp-recorded EEG. However, it is probable that this is a Type II error as a result of the imaging modality. There was a single difference in EP at electrode Pz wherein good navigators exhibited slightly less low frequency activity than did the poor navigators in response to the true stimuli. This is somewhat sensible given the importance of posterior parietal cortex for processing spatial information, but because it is the sole significant difference between good and poor navigators increased scepticism may be warranted.

There are several possible explanations for the lack of significant differences observed between good and poor navigators. Selecting only 5 subjects per group make it possible that the absence of a difference is because the comparison was underpowered. Additionally, the duration of the task was lengthy, so there were relatively few trials in each condition. One or both of these factors could be preventing an effect from achieving statistical significance despite bootstrapping.

Conversely, it is possible that the lack of effect is the result of the complexity of the stimuli being presented in the task. This could be overcome through the inclusion of additional trial types where only the object or only the background scene is presented as a cue. However, this does not examine the process of choosing between the original and the new object location, which is the purpose of these experiments. An alternative approach would be to counterbalance the presentation order such that the object was presented followed by a filling in of the background or the inverse (i.e., background first followed by the object). Again, this was not done because the purpose of the task also involved not

revealing the manipulations to the participant so that they attended to what they perceived to be the most salient factors. Presenting the stimuli separately would likely result in an artifactual inflation of the importance of the background for subjects who would otherwise attend only to the presented object.

Another question raised by the null results found in this chapter is the utility of scalp recorded EEG for studying memory. Associations memory task performance and oscillatory activity in neocortex are detectable via scalp recorded EEG (Hanslmayr & Staudigl, 2014). However, whether scalp recorded EEG is capable of detecting changes in hippocampal activity – particularly when the activity is likely to differ in source rather than timing is an issue that requires careful consideration. Performance on this behavioural task is likely to be hippocampal dependent, but more importantly the subregions that are contributing to task performance likely change with the choices the subject makes. For example, if the subject attends to local vs. global cues, the representation is likely relying on LEC or MEC, respectively. Further, choosing the old location could be argued to be a proxy for pattern completion (i.e., DG dependent) whereas choosing the new pair in the location could be a proxy for pattern separation (i.e., CA3 dependent). In short, high-resolution fMRI or intracranial EEG would likely contribute more to our understanding of the neuroanatomy that underlies task performance than scalp recorded EEG.

Chapter Six

General Discussion

"… so it goes"

-- Kurt Vonnegut, Slaughterhouse Five

This thesis provides support for the theory that sex differences in performance of spatial tasks are an emergent property of differences in how contextual information is interpreted, as well as the strategy implemented during task performance. Several pertinent hypotheses were examined and all but one received partial support. The hypothesis regarding the influence of sex on task performance accuracy was supported. Throughout the literature, sex-related factors are touted as the driver of differences in performance accuracy in males and females (Jonasson, 2005; Kimura & Hampson, 1994). However, our results indicate that males and females may be solving the task differently via the emphasis placed on particular types of cues. Namely, the strategy that individuals implement (i.e., strategy selection) in order to solve the task drives sex differences. Strategy selection appears be the most important factor on task performance. That said, sexually differentiated propensies to implement strategies that were more or less accurate (i.e., allocentric vs. egocentric, respectively) are likely the underlying reason for the sex difference in task performance. This contrasts with the idea that there are important differences in general spatial navigation abilities (Jones et al., 2003). We were able to show that men and women are differentially affected by our manipulations of strategy. Our ability to constrain the strategy that subjects needed to use to effectively solve the real world version of the task provided strong support for the hypothesis regarding strategy selection. The real world navigation experiments also confirmed that

men and women perform these tasks in a similar fashion to subjects in both nonhuman animal studies and virtual navigation paradigms in human subjects (Driscoll et al., 2005; MacBeth & Luine, 2010). The hypothesis that we would be able to distinguish EEG profiles in the time frequency domain between good and poor navigators' responses to congruent and incongruent OL pairs was not supported.

Virtual and Real World Navigation Tasks: Advantages and Disadvantages

The majority of research on spatial abilities in human subjects has relied on tasks where the subject remains stationary such as tabletop adaptations of the Morris water task (Koppen et al., 2013) or object arrays tasks (Saucier et al., 2002). However, movement through peripersonal space tends to rely on regions of parietal cortex rather than hippocampus (Holmes & Spence, 2004), so tabletop versions of tasks may not be approximating the same abilities as tasks that are of real world scale or incorporate the perception of large-scale movement using virtual paradigms. Limited research involving movement through large-scale space has been limited (see Saucier et al., 2002), but the results tend to be consistent with what is observed in virtual tasks (Astur et al., 1998; Driscoll et al., 2005). Though real world movement provides insight into how space is represented in humans, it can be difficult to conduct due to relatively limited abilities to control environmental features, the costliness of space and time required to conduct the research, or the belief by some that virtual tasks do a sufficient job of approximating real world navigation. Consequently, human research on spatial behaviour tends to rely on virtual environments (Astur et al., 1998; Driscoll et al., 2005; but see Saucier et al., 2002), which does have several advantages.

The use of virtual environments creates an opportunity for tight control of experimental parameters. Basic stimulus characteristics such as the field of view, luminance, or colour can be adjusted, landmarks can be added or removed, or distances between points can be reduced or expanded. The ability to manipulate these aspects of the environment allows scientists to explore specific hypotheses and predictions in a way that would be nearly impossible in a real world setting.

An additional advantage of virtual navigation tasks is that they provide an opportunity to keep subjects stationary so that neuroimaging techniques can be used to examine what is occurring in the brain as people "move" through space (Ekstrom et al., 2003). A considerable body of work in nonhuman animals has identified a number of cell types in the hippocampus and related neural regions that correspond to specific types of sensory processing including cells that represent specific locations (O'Keefe et al., 1998), distances to boundaries (Lever et al., 2009), or perceived direction (Taube et al., 1990) as the animal moves through space. Many of these cell types have also been identified in humans through invasive recordings (Ekstrom et al., 2005) or high-resolution fMRI (Ekstrom et al., 2003) during virtual navigation. The observation of analogous activity in humans when stationary provides support for the continued use of virtual paradigms for the continued study of spatial abilities in humans.

Scalp recorded EEG cannot be used to discriminate between good and poor navigators on the virtual reality task

The best and the worst performers on the virtual task were used to examine whether of not scalp recorded EEG responses to the presentation of the stimuli would differ between these groups. The proportion of males and females in each condition did not

differ if the median split was determined using both high and low cue density objects. However, if the groups were populated using only the low cue density objects, females were more likely to be members of the group of poor navigators than their male counterparts.

The absence of a difference between these two groups is likely a Type II error that could have occurred for several reasons. The comparison may have been underpowered by the small number of individuals per group or because it is a between subjects comparison. The lack of effect could also be a result of the complexity of the stimuli being presented in the task. Lastly, scalp recorded EEG collected during the performance of complex tasks that recruit medial temporal lobe may be of limited utility.

Performance on this behavioural task is likely to be hippocampal dependent, but more importantly, the subregions that are contributing to task performance likely change with the choices the subject makes. For example, if the subject attends to local vs. global cues, the representation is likely relying on LEC or MEC, respectively. Further, choosing the old location could be argued to be a proxy for pattern completion (i.e., DG dependent) whereas choosing the new pair in the location could be a proxy for pattern separation (i.e., CA3 dependent). In short, high-resolution fMRI or intracranial EEG would likely contribute more to our understanding of the neuroanatomy that underlies task performance than scalp recorded EEG, which is unlikely to discriminate activity in these deep brain structures, especially when it is the sources rather than the timing of the signals that is important. Regardless, what remains unclear is whether the behavioural difference is a product of the relative importance of brain regions or is due to a difference in the salience of representations in different brain regions.

Differences in spatial abilities are likely driven by sexually dimorphic patterns of recruitment from medial and lateral entorhinal cortices

Attended information appears to be influenced by sex, as indicated by the sex differences in the performance of both the real world version of the Morris water and virtual OL tasks. The results of this thesis are consistent with the literature in that we find that males appear to have more accurate spatial representations of experienced spaces using traditional behavioural neuroscience paradigms for the assessment of spatial abilities. That said, when females are provided with sufficient contextual information, they perform the tasks equally well as indicated by the similarity in performance on the high cue density trials of the virtual task.

The bulk of the literature published on this sex difference in spatial abilities relies on tasks that must be solved using information about spatial geometry. However, our results indicate that the male advantage in spatial abilities may be an artifact of the nature of the information experimenters are providing subjects. There are changes in lateralization associated with memory encoding vs. retrieval and the reliance on verbal vs. other classes of stimuli which could have important implications for the current set of experiments (Davachi, 2006). Interestingly, much of the literature on sex differences in BOLD activity reports differences in lateralization of hippocampal activity, and this has been interpreted as a difference in the nature of attended information or strategy (Frings et al., 2006). It is possible that most of the tasks used to assess spatial abilities are formulated on cues that selectively recruit neural regions that are differentially activated during the performance of spatial tasks. For example, if women are relying on egocentric or local cues in solving spatial tasks, but the experimenters have structured the tasks with Euclidean navigation

strategies in mind, a male advantage will be observed that is corroborated by neuroimaging techniques.

During performance of the real world task, male performance exceeded female performance when we structured the task in such a way that a particular strategy needed to be used in order to successfully solve the task. The starting position-relative goal site used during the third real world experiment likely forced all subjects to rely on an egocentric strategy. In doing so, the performance of the male subjects worsened, whereas the performance of the female subjects did not differ strongly from the females in the first two experiments. Male performance declined when men are forced to implement an egocentric strategy. This finding also implies that females may be using an egocentric strategy as the default to solve tasks under all conditions. Saucier and colleagues (2002) were able to show that female performance degrades when women are forced to implement an allocentric strategy and these experiments reveal that the opposite is also true when males forced to use an egocentric strategy. I propose that this difference is the result of differential weighting of EC inputs to hippocampus and the differences in HPC targets from MEC and LEC (Bush, Barry, & Burgess, 2014; Ferbinteanu, Holsinger, & McDonald, 1999; Henriksen et al., 2010). One possible explanation for the sex difference in strategy selection is the disparate neuropharmacology that characterizes the HPC inputs from EC.

Sex differences in cholecystokinin (CCK) have been reported in rodents, but more importantly, levels of CCK have been shown to alter estrus phase (Vries, 1990). CCK induces long term potentiation (LTP) along the medial perforant path – which is the input to HPC from MEC (Ferbinteanu et al., 1999). Similarly, LTP induction along the lateral perforant path requires opioid receptor activation and sex differences in endogenous

opioid receptor binding and variations across the menstrual cycle have been observed in women (Smith et al., 2006). This is one explanation for why strategy selection would vary across the menstrual cycle in women and the estrus cycle in rodents and how it is contingent on the source of EC inputs.

Differences in the induction of LTP in HPC from LEC and MEC inputs could explain why sex differences in performance accuracy have been reported. Place fields are less spatial in proximal CA1 and place field size increases along the dorso-ventral/proximal-distal axis of HPC (Bush et al., 2014). Inputs from MEC preferentially target proximal CA1 – where place fields are smaller and could be argued to be more refined in space than place fields in more distal regions of CA1 – which receive more input from LEC (Ferbinteanu et al., 1999). The nature of the representation that is constructed from MEC inputs may be more spatially accurate because (1) the information processed in MEC contains more precise, Euclidean information (i.e., velocity, distance) than the context-feature information contained in LEC and/or (2) the targets of LEC exhibit larger – and hence less "accurate" – place fields. Conversely, it is also possible that males and females are equally reliant on inputs from LEC and MEC, but that males are better at integrating egocentric cues about body position with allocentric cues about location relative to the environment.

The performance of male and female subjects on the real world task was consistent with both the animal literature on sex differences as well as the literature on virtual navigation in human subjects (Driscoll & Sutherland, 2005). However, the utility of the real world task may surpass virtual tasks because it allowed for the manipulation of which strategy would be more efficient. Koppen and colleagues (2013) who examined sex differences in this ability using a peripersonal version of the task did not find support, but

this is likely a result of the increased importance of parietal regions in determining body position relative to the role of medial temporal lobe when the whole body is moving through space (or perceiving such movement in virtual reality tasks).

Preliminary analyses (not included) of behavioural data from the real world task revealed that males performed better than females from the first to the second trial. Careful observation of the video recordings revealed that this effect was likely driven by a propensity amongst female subjects to translate the goal location egocentrically (i.e., they would go to where the target location should have been if it was determined using a egocentric frame of reference), according to their starting position. The follow-up real world experiment (Experiment 2) confirmed the prediction that the difference in performance of the male and female subjects on the second trial would transfer to the third trial if the starting location was held constant between the first and second trials, but was moved on the third. The final follow-up experiment was developed to definitively test if males and females were implementing allo- and egocentric strategies, respectively, to solve the task. Forcing subjects to use an egocentric strategy (i.e., the target position is defined by the starting position of the subject rather than a constant position relative to the environment) worsened male performance whereas female performance stayed the same.

The better performance by the male subjects in the first and second studies relative to females may be a reflection of the accuracy of the default strategies that men and women implement to solve the task rather than superior spatial abilities being an inherent trait of being male. Egocentric strategies rely on attention to contextual information that is processed in LEC. The spatial representations supported by LEC lack the spatial accuracy that is found in MEC because of the less-geometric nature of the

information that is processed in LEC (Knierim & Neunuebel, 2015). Conversely, the information computed in MEC is constrained and defined by the geometry of the environment. If this is correct, it predicts that reliance on MEC over LEC would lead to increased accuracy during the performance of spatially demanding tasks because more information about distance, velocity and space are represented in MEC. As a result, geometric or allocentric strategies are more likely to result in increased accuracy of performance on spatial tasks – especially when the contextual information processed in LEC is sparse, such as in the cue sparse condition in the virtual task. A high density of cues in close proximity will make a large difference for a subject who is relying on an egocentric strategy. The same impact will not be seen for subjects relying on an allocentric strategy informed by environmental geometry that uses distal landmarks. This prediction is supported by what we have observed in the performance of both tasks used throughout this thesis and in the work of others who report a male advantage in spatial tasks though most have not accounted for the influence of strategy in task performance.

It is not enhanced spatial abilities amongst male subjects that is reflected in their improved performance of spatial tasks, rather it is improved accuracy associated with the implementation of allocentric strategies relative to egocentric, regardless of the sex of the subject. The reason why males and females differentially implement these strategies is still not understood, but alterations to the default strategy implemented by female subjects across the estrus cycle imply that this difference is mediated by hormones (Korol et al., 2004), though the only relationship between behaviour and hormones was a correlation between levels of estradiol and the duration of the exploration phase of the experiment. Similarly, women's performance on spatial tasks varies with menstrual cycle, pregnancy, and parturition (Macbeth & Luine, 2010). The results of this group of experiments

indicate that male and female subjects solve spatial tasks using unique, but overlapping sets of cues and the parameters of task design can drive the direction of effects. Scientists should carefully consider these factors during the development of future projects as well as how they interpret their results and sex differences already reported in the literature. The existence of sex differences is equivocal, but the underlying causes are not. Assessments of spatial abilities can be designed such that they elicit a male advantage, a female advantage, or no advantage at all.

Evidence for differential recruitment of hippocampus through boundary extension in male subjects.

Males tended to place foil objects outside the navigable area whereas females placed the objects within the boundaries of the navigable area. This may indicate increased confidence, better memory for characteristics of the environment, or an improved capacity for making spatial inferences amongst male subjects. Increased confidence in males regarding their spatial abilities may contribute to the males' willingness to make inferences about position, but this is likely not the sole driver of the differences observed throughout this thesis. Similarly, target accuracy was the same for male and female subjects when there was a high cue density indicating that a difference in memory is also unlikely. An alternative explanation is that boundary extension is sexually differentiated.

Boundary extension is a phenomenon whereby the subject perceives the boundaries of a perceived scene to be further than what was actually presented (Chadwick, Mullally, & Maguire, 2013). When hippocampus has been compromised, boundary extension is impaired (Intraub, 2012; Mullally, Intraub, & Maguire, 2012). The

overall area covered by object placements, including experienced objects, was more expansive for the male subjects. It is possible that the reduced area covered by our female subjects is indicative of increased boundary extension in this group, which may imply that females are relying more heavily on the hippocampus in solving this task. Conversely, it is possible that boundary extension is the product of an egocentric spatial representation.

What does it all mean?

I propose that differences in activation are not necessarily reflective of differences in spatial abilities. Rather, they could represent a difference in the strategy that is being used to solve the task. If males and females are performing a different task at a fundamental level due to attention to different parameters, comparisons of functional activation must duly consider the influence of strategy, especially on patterns of neural activity. The state of the literature on spatial abilities and sex differences is contentious and it is likely that a failure to account for multiple solutions to the same tasks as well as fluctuations in individual performance underlie conflicting results.

The types of information used by male and female subjects both depend on a cortical region located in the medial temporal lobe, the entorhinal cortex – which serves as the major input and output of the hippocampus (Van Strien, Cappaert, & Witter, 2009). Despite relying on the same gross neuroanatomical region, egocentric or stimuli-defined information is thought to rely on the lateral entorhinal cortex (Hargreaves, Rao, Lee, & Knierim, 2005; Yoganarasimha, Rao, & Knierim, 2011). Conversely, processing of environmental geometry (i.e., allocentric representations of space) relies on the medial entorhinal cortex (Knierim & Neunuebel, 2015). Unfortunately, it is not possible to dissociate these two regions with scalp recorded EEG, but a technique such as fMRI

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would likely reveal significant differences in the functional activation of LEC and MEC during the performance of complex spatial tasks such as those used throughout this thesis.

The hippocampus is likely operating in a similar fashion between male and female subjects though it is the integrator of information from these two disparate entorhinal subregions to create a high level representation of the information. However, if a task relies heavily on the use of one strategy or another, it is likely to produce differences in the patterns of activity in regions afferent and efferent to the hippocampus. There may also be differences in activity across cell layers within hippocampus given the differences in termination layers from MEC and LEC (Knierim & Neunuebel, 2015).

Though we were unable to control the strategy implemented by subjects during performance of the virtual task, it is likely that female performance was improved under cue dense conditions because it allows for a more detailed egocentric representation of the space (e.g., "I know the moon is that way because I turned by these rocks"). An additional set of experiments have been developed wherein salient local cues/landmarks can be manipulated or the dimensions of the space can be scaled such that the distance in one direction has been skewed. Using fMRI it would be possible to examine whether males and females are differentially susceptible to these manipulations as well as whether the LEC and MEC respond differently to these manipulations.

The possibility that these two regions will be differentially affected by these manipulations is similar to the examination of whether there would be differences in the EEG profiles of good and bad navigators. The types of information attended to will influence task performance, but more specifically, they will influence the nature of the scenes that have been constructed. When the parahippocampal complex is compromised, subjects lose the ability to imagine scenes (Hassabis et al., 2007; Hassabis & Maguire,

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2009). This finding could be expanded to include predictions about the nature of the scene that the hippocampus is constructing according to the inputs to hippocampus from EC. Hippocampal representations of a spatial scene that have been informed more strongly through LEC inputs is likely to contain fewer precise details about the spatial relationships amongst factors. Conversely, a hippocampal representation that has been informed more strongly through MEC inputs would likely contain more information about the spatial relationships between components of the scenes. Both representations may appear the same at the level of hippocampal activity, but the information contained therein may be quite distinct.

Spatial abilities tend to be defined by the external features of the task (e.g., object location memory, wayfinding) rather than the solutions used to solve them. One could argue that focusing on the solution to a task rather than the ability it is purported to test will lead to a recapitulation of what sex differences in spatial task performance actually means. Strategies may be more or less accurate and males and females may have different pressures leading to tendencies to rely more often on one strategy or another, but this does not mean that one group is better than the other. It is differential recruitment of LEC and MEC and the nature of the information that is processed therein that drives the differences in spatial accuracy and strategy implementation.

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Experimenter 1:
"We are going to start the task now. Once you start, I'm not going to be able to help you, so you are going to have to figure it out on your own. There is a location somewhere in the arena that I can see, but that you and my colleague helping you cannot. It doesn't look different than any other place in the ring, so you won't be able to tell by looking at the spot. When you get to the spot, I will blow a whistle and then you will know that you have found the spot you're looking for. The first time you find it will be random, but we need you to find that spot as quickly as possible, without running. After you find it, we'll get you come back to this start spot, and then we will move to a new start spot, and get you to try to find that target spot again. Each time my colleague will raise their hand once you are ready and I will blow the whistle to signal the start of each trial. Remember, the first whistle is to start the trial and the second one means that you have found that target location. If you hear me blow the whistle twice in a row, it means that something went wrong, and that we have to redo that trial. Does that make sense?"
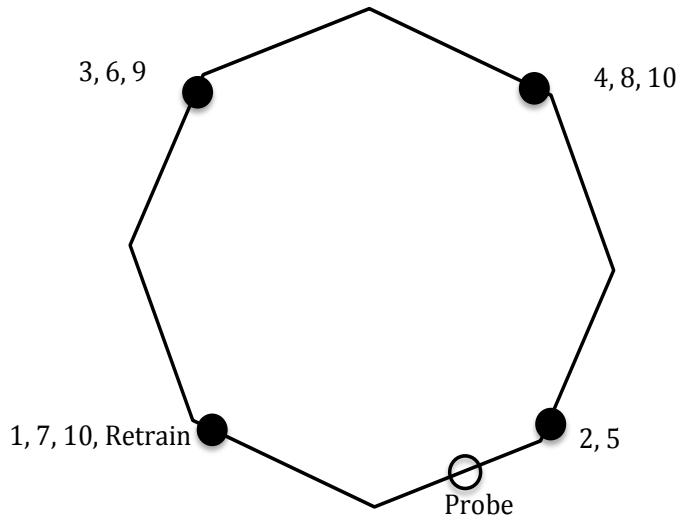
Experimenter 1 will count out the trial number so the next start location is clear.

Start of trial 1: "Are you ready?" <Raise hand so Experimenter 1 knows we are ready> Whistle.
End of a trial 1-10: Say some variation of this:
 "You're doing great! Let's move on to the next trial"

Experimenter 1 will let you know to take the person to the Probe trial start location by mislabelling the current trial number (e.g., "we are now on trial 4, no 5, no 4").
At the end of the 30-second probe trial the whistle will be blown twice.

Please say:
After Probe trial: "It looks like something went wrong with the camera, But I think we have enough data for the day, Great work! " Thank the participant for being so awesome and their participation.

<<Please stop the recording>>

Appendix II: Virtual Experiment Procedures
VR_Nav Experiment Checklist

1. Turn on EEG Data collection computer.
   a. If login required:
      i. username: ███
      ii. password: ███
   b. Launch Netstation > open Session > select 'eye tracker'
   c. Enter subject ID (e.g., VRNav###); ALWAYS USE THIS FORMAT FOR SUBJECT ID
2. Turn on Stimulus Presentation computer, ensure JoyToKey.exe, DragDrop.swf, and UDK game are all launched. Minimize VRGame and DragDrop.
      i. Launch Game. VR Editor > experiment 1.
      ii. Open Display Target Points from StartUp menu.
3. Measure subject head circumference. Place appropriate net in KCl solution while subject completes consent form and questionnaire. Record net use in EEG log.
4. Place electrode net on the subject. Check and adjust impedances on all electrodes as necessary
   a. Lead subject in to testing room. Plug the net in. Turn on secondary monitor.
   b. On EEG computer Measure Net Impedance.
   c. Reduce impedances.
   d. Close Measure Net Impedance Window. Record any bad electrodes in EEG log book.
   e. PRESS RECORD
5. Launch Eyetracker computer.
   a. Launch Eyetrac 6.Net User Interface.
      i. Open windows: ASL Eye-Trac 6. Net User Interface, Eye Discrimination, Head Tracker, Eye/Scene Video
   b. Have subject sit comfortably in chair. Ensure the subject is seated 55-57 cm from the eye tracker unit.
   c. Turn off *Autodiscrimination*.
      i. Restart head tracking. Ask subject to look at the centre point on the display target points screen. Ask subject to refrain from moving around from this point on.
      ii. Right-click and drag inside the POG display until the eye is visible in the Eye/Scene video window. Adjust the Illuminator, Pupil, and CR thresholds until stable the PR and CR are relatively stable.
      iii. Check autodiscrimination.
   d. Calibrate subject gaze.
      i. Calibrate > Standard Calibration. Tell subject that we will begin the eye tracker calibration.
      ii. When subject is focusing on each target, click save current point.
      iii. Open Show Raw Calibration Data. If the calibration is problematic, redo calibration and/or alter the thresholds to ensure that the PR and CR discriminations are accurate.

         iv.   File > New data file – Save subject ID in same format as EEG (e.g., VRNav_001)**

         v.   Ensure that External Data and Auto File Config are set to Autorecord (XDAT bit 7 or 15).

   e. FLIP SWITCH BY EEG AMPLIFIER.

   f. Maximize game screen. Explain to subject that we are about to begin the first phase of the task. Place the joystick in a comfortable position for the subject and press it down to activate the suction cups.

         i.   Press "x" and then "a" to begin the task.

         ii.   At the second screen. Rewet the electrodes.

         iii.   Press "b".

   g. Minimize the game.

   h. Restore DragDrop and take the mouse to the subject. Tell them that this is the last, quick thing they will have to do. Ask them to drag and drop each of the objects to its location in the environment.

6. FLIP THE SWITCH TO END RECORDING.
7. CLOSE DATA FILE ON EYETRACKER. DO **NOT** HIT THE STOP RECORD BUTTON
8. Thank the subject for their participation.

   a. Unplug the net, bring the subject back to the anteroom, and remove the EEG net. Ask the subject if they have any questions. The subject can leave now.

   b. Place the net in the sterilization bucket. Begin the timer (10-mins).

   c. In the finished DragDrop, press the Fn and Print Screen.

   d. Open Paint and press CNTL-V. Save as subject ID.png.

   e. Inside Content Browser > VR Navigation > UDKGame > Logs > Copy file "VR_.log"

   f. Paste into Desktop > gameLog > save as SubjectID.

9. Remove net from solution at end of timer. Rinse net and electrolyte bucket. Rinse net inside bucket with tap water 3 or 4 times. Return to the EEG lab and place net on towel.

<div align="center">Script</div>

"The first thing we are going to do is fill out a consent form. It outlines everything we are going to be doing today. It says 3 hours on the form, but we will only be here for a max of 2. Let me know if you have any questions."

Once the form is filled out.

"Because we are using the saliva to look at levels of hormones we need you to rinse out your mouth. I have a bottle of water for you. Please take a small sip and use it to rinse out your mouth. Any food particles or blood will contaminate our sample, so we need your mouth to be as clean as possible. Our last step before we head down to the EEG suite is to fill out the anonymous questionnaire. We've set it up online so it's only ever saved with this random ID number and the only people with access to both are Erin and Rob. We won't look at your responses until we have collected all the data so we won't remember

that you are this number. Feel free to answer honestly as it is confidential and anonymous. I am going to go set some stuff up in the other room. I'll be back shortly if you have any questions."

Take the water bottle with you to the EEG suite. Lead the subject to EEG and have them sit in the chair. We are going to put on the net. Give them a towel to keep dry/dab their face. Hand them the plug for the net.

"I'm going to be putting the net on now. There are 128 sensors and each has a small sponge that has been soaking in a saline and baby shampoo solution and a tiny silver wire that we use like an antenna to pick up the electrical activity at your scalp. Most of the sensors will be in your hair, but there are a few that are on your face so I'm going to need you to close your eyes while I put the net on and keep them closed until I ask you to open them. I'm going to start at the back and end by tightening these beads under your chin."

While adjusting the net, begin collecting saliva sample.

" While I'm touching up the electrodes I'll get you to start on the saliva sample. You're going to want to let saliva pool in the bottom of your mouth and then drool it into the tube. We can't analyze foamy spit, so try to do it gently. We need you to fill it to between the 2 and 3 mark".

Position subject for eye tracker.

"I'm going to start adjusting the eyetracker. Please look at the centre dot passively so your pupil is in the optimal position for determining the lighting levels. I'll be right outside getting that set up"

Calibration. "We're going to calibrate the eyetracker now. Imagine the dots are like the number pad on a phone. You have 1, 2 3 across the top, 4, 5, 6 across the middle and 7, 8, 9 across the bottom. When you hear me say a number I need you to look at that dot until I tell you to move on."

Task. "We're going to start the task now. You will be in a large grassy field bordered by a white fence. Please stay inside the fenced area. There are several objects scattered throughout the environment. They are super bright, large, and obvious so you'll know them when you see them. The objects are going to appear when you are a certain distance away from them. When you find an object, you need to move toward it to collect it into your inventory. You do this by walking over the object. You will hear a tone and this means that you have added that object and can continue searching for others. It will still appear though you only need to collect each object once. You are going to need to remember where you encountered the objects because we will be testing your memory for the objects later. You move through the environment using the joystick. You can move left, right, and you can walk or run. You walk by tilting the joystick partially forward and you run by tilting the joystick all the way forward. To stop moving pull back. If you want to go back to the start location, press the top button. If you haven't found a new object for several minutes, I will let you know that I am going to move you to the general area of an

object so you can find as many objects as possible before we run out of time. Do you have any questions?"

Task: Trials Phase. "How are you feeling? We are about to start the memory portion of the task. The most important thing to remember is that the objects are not going to appear, even if you are in the right spot. Each trial will begin with a crosshair followed by a picture. What you need to do is travel toward what you saw in the picture. You're going to do this by rotating left or right until you are facing the optimal direction and then as quickly and directly as possible you need to walk to your target. Once you reach where you think you're supposed to be, you're going to press the front trigger button and that will start the next trial. There are 100 of these in total, but we will have a break after 50. Do you have any questions? … So remember, rotate until you are facing the optimal direction and then travel to your target as quickly and directly as possible and press the front button to move on. Good luck" Press B

At break screen (after trial 50). "Half done. Keep it up." Retouch electrodes. Press L.

Object Placement Task. Launch drag/drop game. "This is the last thing we are going to do today. It's an easy puzzle game. You are going to drag the objects to where they go in the environment using this mouse. Click the object, drag it to where you think it should go and let it go. Try to align the little x with the exact site. You can move them around until you are satisfied."

Task complete. Thank and debrief the subject.

Appendix III: Task Development: Engine Specs and Design

Toggle Trials/Freeroam

Teleport
In
Target   Destination

Level Loaded
Loaded and Visible

ToggleObs
Toggle
Turn On
Turn Off   Out
Target   Bool

CheckFreeroam
Compare Bool
In   True
          False
          Bool

All ..yers Play..rt_1

Toggle Actors
Toggle Hidden
Hide
UnHide
Target

Matinee
Play
Data   Player   Targets

???

True

ToggleFlag

Star
Inte..r_11 Inte..or_2 Inte..or_3 Inte..or_8
Diamond Cone

Coin
X Moon Parallelo Hexagon
Inte..r_10 Inte..or_9 Inte..or_6 Inte..or_7

Mati...0s)

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